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THE CHEMICAL PROPERTIES OF *DERRIS ELLIPTICA* (TUBA ROOT)

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(With 1 Text-figure and Diagram I.)

HISTORICAL.

TUBA root first interested Europeans as being a constituent of the "ipoh" arrow poison of the Malays (1) and of the "siren" arrow poison of Borneo (2). For this purpose the green root was macerated with water, the arrow head alternately painted with the resulting milky fluid and dried in the sun until a sufficiently thick coating of poison was formed.

Latterly its chief use has been as a fish poison; though at present such use is prohibited. It is now employed by Chinese market gardeners as an insecticide and there is a growing demand for it on the English market.

BOTANICAL.

Tuba root (*Derris* (*Pongamia*) *elliptica* Benth.) belongs to the papilionaceous legumes, which include many intensely poisonous plants such as Pongamia, Millettia and Tephrosia, all nearly allied to Derris. A botanical description of the many closely related species of this plant is given in Brandis' *Indian Trees*. As early as 1848 Oxley (3) used a decoction of the root as a means of controlling pests of the nutmeg tree. Attention was drawn to the root again in the *Kew Gardens Report* for 1877 (4) where it was suggested as an insecticide. In 1886 it was exhibited in the Straits Settlements Courts of the Colonial and Indian Exhibition at South Kensington. The desirability of chemical and physiological examination and its possible use as an insecticide were pointed out in the *Pharmaceutical Journal* (5). Wray, in 1882, stated that the Chinese market gardeners were using it for this purpose. The root was chopped up fine in the fresh state, then pounded and mixed with water. The resulting milky liquid was sprayed or brushed over the plants with a bunch of feathers.

The report in the *Pharmaceutical Journal* of the Colonial and Indian Exhibition, referred to above, led Greshoff(6) to undertake a chemical examination of the root. He extracted the coarsely-ground root bark with water in a percolator to free it of tannins and mucilage. After drying, it was extracted with alcohol; to the extract water was added to precipitate the resinous poisonous principle. The alcohol was evaporated off, leaving the "derrid" in a semi-fluid state under a milky supernatant fluid. This crude derrid was purified by treatment with dilute alcohol, followed by 5 per cent. alkali; it was then dissolved in chloroform, washed with water, the solution digested with animal charcoal, and concentrated, whereupon yellow acicular crystals separated out. The purified derrid separated out as a resin on evaporating off the chloroform. It could be fractionated by dissolving in pyridine and adding petroleum ether gradually, the most highly coloured portions separating out first. Greshoff however failed to isolate any white crystalline product. He points out that the poisonous principle is neither a glucoside nor an alkaloid. He describes it as a resinous body of acid reaction, M.P. 61° C. (crude substance) which decomposes at 160° C. giving off an odour resembling coumarin. On heating with caustic alkali it yielded salicylic and protocatechuic acids. He also found that one five-millionth part of the resin stupified goldfishes in a few minutes and killed them in less than half an hour. Thus it is one of the most violent fish poisons known.

Wray junior(7) separated the toxic constituents by warming the chopped and crushed root with alcohol acidified with hydrochloric acid for some hours, filtering and evaporating on the water bath at a low temperature until a gummy substance separated. This he named "tubain." When all the alcohol was evaporated, the tubain was removed and washed by kneading in hot water and further purified by re-solution in alcohol and repeating the above process. A dirty white crystalline substance separated out from an alcoholic solution of tubain on standing. This substance, he states, was not poisonous to fish when freed from tubain. The poisonous principle was not an alkaloid. It gave a dragon's blood red solution with nitric acid. Wray found that ·03 per cent. of the green root in water sufficed to kill fish. Some of the physiological effects on fish are mentioned in the paper. ·00029 per cent. of the resin was quickly fatal to fish, while ·00001 per cent. killed in from 15 to 30 minutes, according to the species. From the fact that fish may eat fairly large amounts with impunity, he argues that the insolubility of the substance in water, renders it necessary to use it in the form of an emulsion, in which form doubtless it exists in the milky sap of the plant. He suggests that we follow the lead of the Chinese gardeners and use it as an insecticide. For this purpose Wray points out the dried root would be of little or no use. The poison would have to be extracted and converted into an emulsion or into some chemical combination easily dissolved in water.

In 1889 Sillevoldt (8) published the results of his chemical investigations. He extracted the root with water, dried, then extracted with boiling 96 per cent. alcohol. The resinous product was dissolved in ether and precipitated with petroleum ether. He states that if the resin be boiled with alcoholic hydrochloric acid the anhydroderivative $C_{33}H_{28}O_9$, $\frac{1}{2}H_2O$ is obtained. This is contained in the original resin "derrid" which has a formula $C_{33}H_{30}O_{10}$ when pure. The anhydroderivative crystallises in fine light yellow needles, M.P. 214° C. Both the above compounds contained three (OCH_3) groups. By treating these with hydriodic acid a body of constitution $C_{30}H_{19}O_6(OH)_3$ was obtained, M.P. 240° C. He was unable to prepare the acetyl or benzoyl derivatives. Sillevoldt apparently failed to isolate the white crystalline product, or none was present in the sample on which he worked. The anhydroderride was not found poisonous to fish.

Power (9) dealing with the stem of a closely allied plant, *Derris uliginosa*, makes some interesting observations in regard to its poisonous action towards fish.

In 1916 Campbell (10) published work from the physiological standpoint. An interesting point in his paper is that the root acts as a stomach poison towards monkeys.

Ishikawa (11) investigated a white crystalline product, isolated by him from the root, which he named "tubatoxin," though the name toxin is hardly warranted by his observations, as the compound is not a protein and there appears to be no evidence of it having the property of inducing the formation of an antitoxin after administration to an animal. (The original paper however was not accessible to us.) The crystals vary in shape but are mostly hexagonal or acicular, soluble in various organic solvents, M.P. 163·3° C., formula $C_{18}H_{18}O_5$, not hygroscopic, burn with a peculiar odour, when heated on platinum foil, leaving no ash, reduce ammoniacal silver nitrate and alkaline copper sulphate, not affected by cold concentrated alkalis, but destroyed by concentrated sulphuric acid.

He gives the following test for the substance. The solution in glacial acetic acid gives a yellow colour on the addition of one or two drops of fuming nitric acid, followed by dilution with water. When the resulting solution is added to dilute sodium hydrate a ring appears in the line of contact coloured green above and reddish brown below. This reaction reveals the presence of ·001 gm. of tubatoxin. We find that a similar colour reaction is given by the resins, although the actual shade is not identical with that shown by tubatoxin. The symptoms produced in animals (fish, rat, frog, rabbit and dog) were general motor paralysis with dyspnoea of central origin. Blood pressure was first raised, then markedly depressed and palpitation of the heart gradually ceased. When injected intravenously the minimum lethal dose for the rabbit was ·0009 gm. per kilogram of body weight. On subcutaneous injection, tubatoxin was absorbed with difficulty.

The most recent paper on the subject is that of McIndoo, Sievers and Abbott (12) who treated it from the entomological and practical sides only. Their conclusions were:

1. *Derris elliptica* and *Derris uliginosa* act both as contact insecticides and as stomach poisons, affecting different classes of insects according to the development of their nervous systems.

2. Denatured alcohol was found to be a good economic solvent for extracting the toxic principle, which, when applied in spray mixtures, proved to be efficient against certain aphids, potato-beetle larvae, and small fall webworms.

3. Derris used as a dust under practical conditions was found to be efficient against dog fleas, chicken lice, house flies, three species of aphids (*Aphis rumicis* L., *A. pomi* De Geer, and *Myzus pessicae* Sulz.), potato-beetle larvae, and small fall webworms, but of no practical value against bedbugs, roaches, chicken mites, mealybugs, orthezia insignis, red spiders, or against the crawling young of the oyster shell scale. Used as powder in water with or without soap under practical conditions, it proved to be efficacious against most of the aphids sprayed, and also against cabbage worms (*Autographa brassicae* Riley), the larvae of apple datuns (*Datana ministra* Dru.), oak worms (*Anisota senatoria* S. and A.), small tent caterpillars and potato-beetle larvae.

After the work for this paper had been completed, we were favoured with notes on investigations carried out between 1902 and 1907 by Dr H. E. Durham; for commercial reasons his results have not been published. In general, Durham's conclusions in regard to the insecticidal properties of Derris are concordant with ours; thus larvae of lepidoptera and sawflies are very susceptible, and aphides (*Bean aphis*, *A. rumicis* and *Woolly aphis*, *E. lanigera*) quite resistant to wet application. Mosquito larvae and pupae, fresh water crustaceans and molluscs, also tadpoles and fish all formed usefully susceptible test animals. Durham insists on the existence of the active principles in a crystalline and a resinous form (cf. van Sillevoldt), and states the former is best obtained from the original petrol ether extract freed from resin first by cold absolute alcohol and then repeated crystallisations from the same solvent heated, until the laminar acicular crystals of constant M.P. at 164½° C. are obtained. Both of these active principles give (*pace* the impossibility of getting a crystal-free resin) a characteristic and intense red colour reaction with strong nitric acid which contact with a drop of ammonia changes to an evanescent deep peacock blue-green. Tests for nitrogen, sulphur, glucosides and cholesterols (*v.* Power) proved negative. Owing to lapse of time and loss of some notes further details of other chemical and biological trials (frogs' hearts etc.) cannot be given here. As regards insects he concludes that toxic effects are due to absorption from the alimentary canal rather than from surface contact.

EXPERIMENTAL PART

SEPARATION AND DESCRIPTION OF CONSTITUENTS.

The root was extracted with 95 per cent. alcohol. Owing to the chemical change undergone by tubatoxin on boiling with this solvent, it was found necessary to carry out the process at a temperature not exceeding 30° C. Large amounts of the powdered root had to be extracted, and as the usual methods at our disposal entailed considerable labour and large losses of solvent, a suitable automatic percolator was devised, of which Fig. 1 is a diagram.

The container, *A*, into which the powdered root is placed, is an inverted bell-jar. Another similar jar, *B*, is placed over *A*, an annular rubber ring making an airtight joint between them. It is a convenience to have a tubulure and tap *D* in *B*, but a T-piece and a tap between *C* and *B*, would be effective. A two-litre round-bottomed flask *F*, the neck of which has been cut off short, is connected with the bottom of *A* by tube *E*, and with top of condenser *C* by tube *J*, which should have a sufficiently wide bore to allow any condensed alcohol to flow freely back into the flask *F*. Bumping is prevented by the introduction of a capillary *P*. Tube *H* and funnel *G* are used for the withdrawal and introduction of liquid. *F* is partially immersed in a water bath, kept at a constant temperature of 30° C. by means of a thermo-regulator. To fill the apparatus it is disconnected at *L*, and *B* and *C* taken off together. The rubber ring is removed. A suitable filter is placed in the neck of *A*; if the material is coarse, a plug of glass wool is sufficient, if fine as in our case, teased-out cotton wool must be used. The rate of percolation can be increased through the enlargement of the filtering surface, by placing sand above the wool to about *M*. The substance to be extracted is placed in *A*. The top rim of *A*, the bottom one of *B*, and the rubber ring are thoroughly cleaned, and the apparatus set up as shown in Fig. 1, connection made at *L* and the whole apparatus evacuated through *D* to get rid of the air enclosed in the capillaries of the powder. When the apparatus is evacuated, alcohol is introduced through *D*, by suction. After the alcohol has percolated through the powder it is necessary to complete the evacuation of the apparatus. To preserve a good vacuum in the apparatus, it may be necessary to smear the outside of the rubber ring with vaseline or other thick grease. There is practically no danger

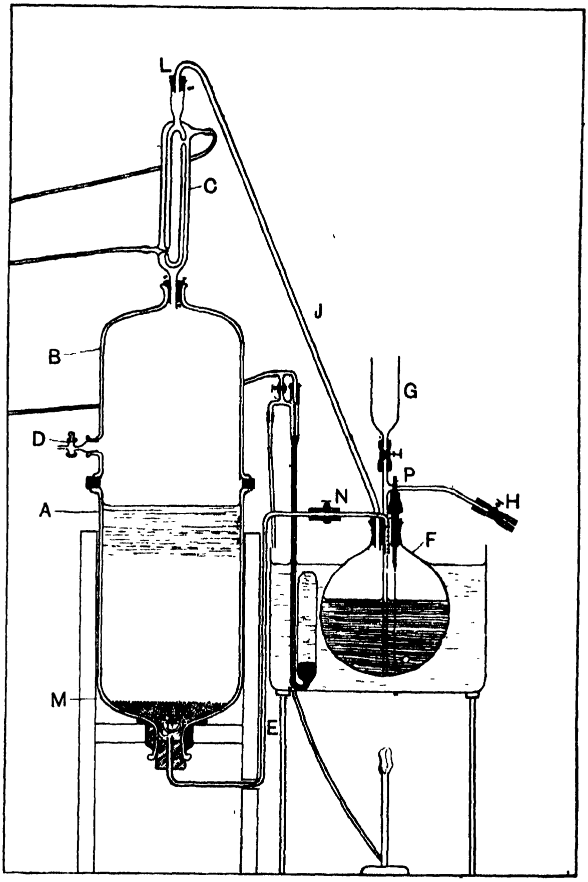

Fig. 1.

of the vaseline being dissolved by the solvent if put on after the apparatus has been set up. The filtered alcoholic extract passes via tube *E* to the flask *F*. The alcohol boils under the partial vacuum in *F* and the vapour, after passing up the tube *J*, condenses in *C* and drips into *A*. In our procedure when the extract became concentrated in *F*, fine crystals separated out. These were removed by evacuating through a filtering flask at *H*, the crystals filtered off and the extract returned through the funnel *G*. On completion of the extraction the highly concentrated extract can be removed by suction through *H*, after which the excess of alcohol in *A*, can be syphoned off into *F* and through *H* by suction, the process being much speeded up by removing *B*, closing tube *J* and placing weights upon the extracted powder in *A*. The last traces held up by the powder, were removed by transferring to a steam jacketed vacuum pan, to which was attached a condenser. By adopting this practice very little alcohol need be lost. The apparatus with us proved very convenient; it may require evacuating occasionally, but otherwise it runs automatically.

The highly concentrated extract is then filtered and the mass of impure crystals, and deep brown solution so obtained, treated separately. The crystalline product is best purified by drying in the steam oven, after being filtered free from alcohol. This drying seems to render insoluble some of the dark viscous material with which the crystalline mass is contaminated. In cases where the original product was very impure, we found that the best procedure to adopt was to dry the crystals from benzene crystallisation, moisten with alcohol, re-dry in the oven and then proceed with the recrystallisation from benzene. This last procedure however was only found to be necessary when from any cause the extraction apparatus was allowed to stand a long time after the extraction was complete, in which case the crystals were cemented by resinous matter. In this way we were able to isolate "tubatoxin," a white crystalline product previously obtained by Ishikawa (M.P. 163° C.) and two yellow crystalline products, one of which corresponded to the anhydroderride of Sillevoldt, M.P. 214° C. Both these products are distinctly less soluble than "tubatoxin." Care and speed have to be exercised in the recrystallisation, particularly if alcohol be used, as the "tubatoxin" readily undergoes change, a dirty brown product being the result. This product on fractional crystallisation was resolved into a resin (which can be fractionated to some extent by precipitating its ether solution by pentane or petroleum ether), and three yellow crystalline products. Two of the latter correspond to the two isolated from the extract of the root, while the other had a melting point of

222–224° C. It seems probable therefore that the yellow crystals isolated by us, and by Sillevoldt directly from the root extract are products of chemical change undergone by "tubatoxin" during the process of extraction. Sillevoldt describes the preparation of anhydroderride from the resins isolated from the root, by boiling them with alcoholic hydrochloric acid; but we were not able to confirm this, provided that pure resin free from "tubatoxin" was used; but the change undergone by "tubatoxin" on boiling with alcohol goes smoothly and easily. The supernatant liquid filtered from the crystalline products was treated with basic lead acetate, only the quantity necessary to precipitate tannins, gums etc., being used, as it was found that the resins themselves are brought down by an excess, and their recovery was a matter of some difficulty; we had evidence also that the product derived from the lead acetate precipitate of the resins, was different from the original resins. The lead precipitate was separated and the filtrate afterwards aerated to free from sulphuretted hydrogen, the alcohol distilled off *in vacuo*, taken up with ether, filtered from any crystals, and precipitated by pentane in fractions. The final fraction soluble in ether-pentane, evaporated at ordinary temperature, was thoroughly extracted with pentane to free from "tubatoxin." This solution on evaporation gave a small quantity of liquid resin which could be partially purified from "tubatoxin" by taking up with a small quantity of pentane, cooling in a solution of solid CO_2 in ether, and filtering while being cooled in the same medium. The oil was never freed entirely from "tubatoxin," as we had only a few drops on which to work at any one time. The resins which were golden yellow in colour had the following melting points.

Fractions:	(1) 70–90° C.	(3) 62–76° C.
	(2) 72–82° C.	(4) 60–64° C.

All these resins and the oil proved toxic to insects, the oil however probably owed its toxic properties, at any rate to some extent, to the small amount of "tubatoxin" from which we were never able to free it. The dark brown lead precipitate was worked up by suspending in alcohol, saturating with H_2S, filtering and after aeration evaporating to dryness. A brown amorphous product resulted. It was non-toxic to insects. An ultimate analysis of "tubatoxin" and the resins, showed only the presence of C, H and O. The most characteristic feature of the crystalline products and the resins, was the presence of methoxyl groups. The fact that "tubatoxin" is readily converted into a resinous substance, partially precipitated from ether solution by pentane and of a melting point 62–72° C.

and into crystalline yellow derivatives indicates that all these products are inter-related and probably have a common derivation. In a schematic form the process is expressed in Diagram No. 1, in which toxic constituents are indicated by means of heavy lines.

SEPARATION OF CONSTITUENTS OF TUBA ROOT.

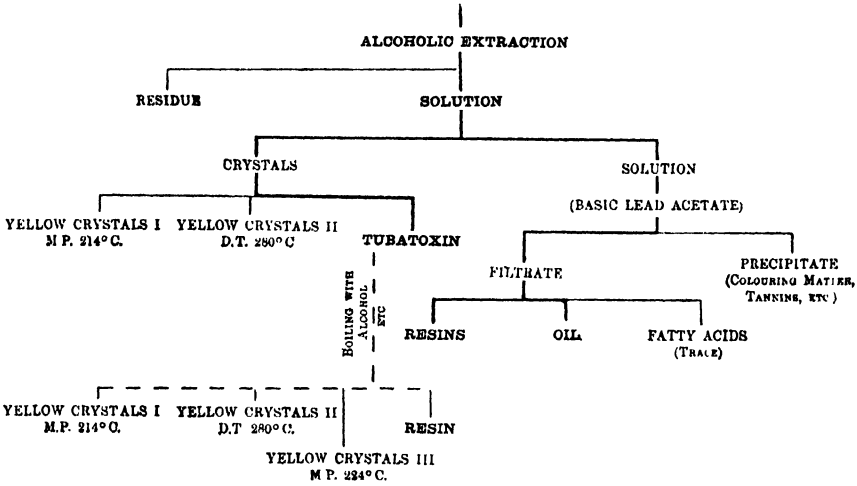

Diagram 1.

PROPERTIES OF THE CONSTITUENTS OF THE ROOT.

"*Tubatoxin.*"

(1) Colourless crystals.

(2) Soluble in most organic solvents, insoluble in water.

(3) Forms molecular compounds with various organic solvents, notably benzene and alcohol.

(4) Its ether solution not precipitated by pentane.

(5) Contains only carbon, oxygen and hydrogen.

(6) Heated to 100° C. in a current of oxygen or carbon dioxide resinifies, becomes brown in colour. The rate of change is slower in carbon dioxide.

(7) Contains 15·4–15·6 per cent. CH_3O.

(8) Alcoholic solution reduces ammoniacal silver nitrate.

(9) On boiling with alcoholic caustic potash, turns deep red in colour, and gives rise to a saponifiable and an unsaponifiable portion—both are resinous.

(10) On boiling with alcohol (95 per cent.) gives rise to a series of

yellow crystalline derivatives and to a resin melting at 62–72° C. A trace of moisture accelerates this change.

(11) Undergoes chemical change when its solutions are exposed to sunlight or ultraviolet light.

(12) Is toxic to insects.

Nos. (3), (6), (10) and (11) are considered in detail below.

(3) *Solvent of crystallisation of "Tubatoxin."* Small samples of crystals from benzene were warmed in a sprengel pump vacuum, and crystals from benzene and alcohol dried in the steam oven with the following results. 129·3[1] parts of crystalline product from benzene gave 100 parts pure "tubatoxin" on drying three weeks in Sprengel vacuum. 119 parts of crystalline product from benzene gave 100 parts pure "tubatoxin" on drying one week in steam oven. 103 parts of crystalline product from alcohol (needles) gave 100 parts pure "tubatoxin" on drying to constant weight in steam oven. 100 parts of crystalline product from alcohol (plates) gave 100 parts pure "tubatoxin" on drying to constant weight in steam oven.

From the Sprengel pump vacuum test we were able to collect a little clear liquid which had a boiling point of 80° C. and melting point of 4–5° C. and answered the tests of benzene.

These experiments show that we have definite proof of one crystalline product with benzene of crystallisation and of one with alcohol (needles). The plate crystals from alcohol appear to be free from solvent of crystallisation.

(6) *Effects of heat on the toxic constituents of the Root.* "Tubatoxin," resins and oil were weighed out into porcelain boats, and kept at 100° C. for some days in an atmosphere of oxygen and a control set kept at the same temperature in carbon dioxide.

The loss was less in each case in oxygen than in carbon dioxide. "Tubatoxin" in CO_2 gave rise to a product coloured purplish-brown. "Tubatoxin" in oxygen gave rise to a product coloured brown. Under the microscope the latter showed crystals changed in places to a yellow product. The resins appear to suffer no loss of solubility by heating in carbon-dioxide but suffered very remarkably in oxygen. There was much greater darkening in colour in oxygen than in carbon dioxide. The oil darkened in colour in each case but also resinified in oxygen.

(10) *The change of "Tubatoxin" on boiling with alcohol.* This has already been briefly considered; it has not been completely worked out, but is full of interest. Traces of moisture accelerate it. "Tubatoxin" solu-

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[1] Owing to an accident it was impossible to continue this to constant weight

tions in absolute alcohol do not change in colour on boiling so readily as do those prepared with 95 per cent. alcohol. Equal quantities therefore of the same colourless solution of "tubatoxin" in alcohol were poured into two tubes. To one was added a few drops of water and they were heated on a water bath. After half an hour the tube containing water was markedly more yellow than the other, which remained practically colourless.

(11) *Colour Change of "Tubatoxin."* We have already seen that on exposure to light an alcoholic solution of "tubatoxin," or more so on boiling, gives rise to a yellow solution (p. 9). It was observed that dry crystals of the substance stored in a tube became a pronounced yellow on the side of the tube more exposed to the light, without suffering any appreciable change of colour in sheltered parts of the tube. As it was necessary to carry out insecticidal tests with this material in as pure a condition as possible it was considered desirable to ascertain how rapidly this colour change could be induced. A 4 per cent. solution in carbon tetrachloride which was colourless when made up became a reddish-yellow on standing in the light for some weeks. A slight reddish-brown precipitate was also formed. This solution was filtered and 20 c.c. introduced into each of the receptacles of the Duboscq colorimeter. No. 1 was kept in the instrument where it was screened from any direct light, and No. 2 was exposed to the light from a mercury lamp. After two hours and four hours respectively, their relative colour intensities were measured, with the following results:

	Depth of liquid in tube		Intensity of colour 1 / Intensity of colour 2	Mean
	1	2		
After 2 hours	3·0	2·83	·94	·85
	2·5	2·07	·83	
	2·72	2·59	·92	
	3·10	2·44	·80	
	3·10	2·72	·88	
After 4 hours	2·27	1·60	·70	·67
	2·0	1·38	·69	
	2·0	1·23	·62	

Hence there was a marked increase in colour on exposure to the light from a mercury lamp.

Resins.

1. Yellow to light brown in colour.
2. Precipitated by an excess of basic lead acetate to give a light brown product.

3. Readily soluble in most organic solvents, with the exception of pentane, petroleum ether and water.

4. Partially precipitated from methylated ether by pentane and petroleum ether. It can be partially fractionated in this way. The following fractions were thus obtained by us:

A 1. Dark coloured, very small quantity, M.P. 70–90° C.
A 2. Light yellow, M.P. 72–82° C. methoxyl content 16·0 per cent.
A 3. Light yellow, M.P. 62–76° C. methoxyl content 15·3 per cent.
A 4. Soluble in pentane ether. Insoluble in pentane, M.P. 60–64° C. methoxyl content 13·8 per cent.

5. Poisonous to insects.

Liquid resin.

1. Light yellow coloured oil, soluble in pentane.
2. Partially resinifies on standing.
3. When purified as far as possible, has a methoxyl content of 2·85 per cent.; this was probably due to presence of traces of tubatoxin, as on progressive purification the methoxyl value decreased in amount from 4·6 to 2·85 per cent.
4. Darkens in colour and resinifies on heating at 100° C. in oxygen.

Yellow Crystalline Derivatives.

1. Three of these were separated having melting points of 213–214° C. 224–226° C. while a third set of crystals decompose at 280° C. without melting.
2. Crystallise in long needles.
3. They are all only slightly soluble in organic solvents.
4. Methoxyl content 16·6–16·8 per cent.
5. Do not precipitate silver from alcoholic ammoniacal silver nitrate.

There was a wide variation both in the total amounts, and the relative proportions of the various constituents of the roots. Some consignments contained as much as 20 per cent. of poisons while in others it fell as low as about 5 per cent. The non-poisonous constituents also varied considerably. Moreover in some of the roots the amount of tubatoxin present was not great while in others it formed one-third of the toxic constituents.

The approximate yields of the various constituents not precipitated by basic lead acetate in the poorest quality of root submitted to us are given in Table I.

In the second experiment the resins were slightly contaminated and the amounts of pure resin were calculated from their methoxyl contents.

Taking the figures in the first set and the amount of each constituent and its CH_3O value, the calculated value for the CH_3O content for the whole extract of poisons is 14·6 per cent.

An extraction with carbon tetrachloride gave 4·73 per cent. of extract with a methoxyl content of 14·7 per cent.

Table I.

	Experiment 1		Experiment 2	
Constituent	% on root	% CH_3O in constituent	% of impure constituent on root	% pure constituent on root
Tubatoxin	1·48	15·65	1·33	1·33
Resin precipitated from ether solution by petroleum ether 2 : 1	1·29	15·79	1·78	1·37
Resin soluble in ether and petroleum ether 2 : 1	1·39	13·94	1·23	1·09
Liquid resin	0·22	4·6	0·29	0·29
	4·38		4·63	4·08

THE INVESTIGATION OF A METHOD OF EVALUATING THE ROOT.

The results obtained by the approximate analysis of the root and the investigation of the various constituents indicate a ready method for evaluating the root by chemical means. This is very necessary as the different samples tested by us were found to vary widely in their toxicity to insects and the methods of quantitatively testing them for their insecticidal properties is both difficult and tedious, and have only been partially investigated and moreover are likely to give results of differing value according to the type of insect chosen, the number available, the season of the year and the method of application. Three different consignments of root were examined, they are labelled for convenience *X*, *Y* and *Z*.

Derivation of *X*. Penang.
„ „ *Y*. Kuala Lumpur.
„ „ *Z*. Kuala Lumpur.

They were carefully sampled and the soil detached by picking over, and ground to an almost impalpable powder.

Moisture content. As the products of the powdered root undergo chemical changes on warming, it was found necessary to carry out this estimation at the temperature of boiling dichlorethylene, B. Pt. 55–58° C.

in partial vacuum, over P_2O_5. Even at this low temperature some slight chemical change took place.

Results:	Root *X*.	(1) 8·64	(2) 8·61
	„ *Y*.	(1) 8·50	(2) 8·54
	„ *Z*.	(1) 9·65	(2) 9·49.

Five grams of the finely divided root were weighed into glass vacuum drying bulbs containing glass stoppers fitted with sealed-in glass tubes into which light plugs of cotton wool were placed, one tube being closed by a rubber tube and glass stopper the other being connected through two flasks in series containing P_2O_5 to the water pump. They were kept thoroughly evacuated and dried till the weight reached a minimum.

Conditions necessary for the Estimation of Toxic Extract.

This is based upon the extraction of the powdered root by dry, alcohol-free solvents, preferably ether. Difficulties were encountered in working out this apparently simple method, widely varying results being obtained for the quantity of the extract according to the solvent used, the degree of fineness of the root, the amount of moisture present in the root, the temperature of the extraction and the way in which the extract was dried. These variations are due to a number of factors.

(1) The extraction must be selective.

(2) The root is very impermeable to solvents and so must be ground to an almost impalpable powder, moreover if this is not done there are difficulties in weighing out a true representative sample of the root.

(3) The root must be extracted at fairly low temperatures, as otherwise chemical changes take place which may give rise to sparingly soluble compounds.

(4) The extract undergoes chemical change on drying.

(5) The type of extraction flask may modify results.

(6) The toxic constituents are freed from the last traces of solvent with great difficulty, as illustrated by the case of "tubatoxin" (p. 10).

Difficulties 4, 5 and 6 can be illustrated in the case of the carbon tetrachloride extract of a sample of root. Short drying in vacuum desiccator plus 1½ hours at 100° C. in round-bottomed flask. Per cent. extract on dry root = 5·27.

Prolonged drying at 100° C. in round-bottomed flask. Per cent. extract on dry root = 4·92.

Prolonged drying at 100° C. in flat-bottomed flask. Per cent. extract on dry root = 4·51.

Difficulty No. 4 proved insuperable and it was decided to dry as rapidly as possible to constant weight at 100° C. using a flat-bottomed flask, keeping conditions as far as possible constant throughout. After trials with several organic solvents, ether dried over anhydrous calcium chloride and sodium was finally found the most satisfactory, as it is very volatile at low temperatures, and is selective in extraction.

Apparatus. Soxhlet extraction apparatus was used, with Davis double jacketed condenser, flat-bottomed flasks, all the joints being of ground glass. The best form of heating apparatus is an electric hot plate, a thick asbestos sheet being interposed so that the ether only gently simmers, it should not be allowed to boil violently. The extraction flask could be readily converted into the Perkin methoxyl determination apparatus by means of a long accurately ground-in glass neck with side tube, down the centre of which ran a narrower glass tube for carrying CO_2.

METHOD PROPOSED FOR EVALUATING THE ROOT.

The finely powdered root is extracted with dry ether in the Soxhlet apparatus as described above, the extract dried at 100° C. and weighed to constant weight.

Hydriodic acid (S.G. = 1·7) previously heated to 120° C. in a current of CO_2 to free from H_2S is added in an amount appropriate to the weight of extract and 5–10 c.c. of acetic anhydride slowly run in and the methoxyl content determined by the Perkin method (13). Drying of the ether extract should not be too prolonged, as the extract itself is normally only very slowly acted upon by the hydriodic acid, prolonged drying at 100° C. tends to accentuate this. The determination of the methoxyl content usually takes two or three hours, depending upon the amount of the extract treated. Collected results are shown in Table II.

Table II.

Root	Moisture content %	Extract % on root	Extract mean % on moisture-free root	Methoxyl content % on ether extract	Alcohol extract after ether extract %
X	8·62	22·1	—	—	—
		21·4	23·7	14·7	—
		21·7	—	14·77	4·1
Y	8·52	17·89	—	14·02	—
		17·44	—	14·7	8·07
		17·80	19·2	—	—
		17·36	—	14·0	—
Z	9·57	7·10	—	13·43	11·8
		7·15	7·9	13·5	–

The difficulties of obtaining closely concordant results are illustrated in Table II, but the results are sufficiently near to indicate that the method will serve for standardisation if the root is to be employed as an insecticide. This will be dealt with under the discussion of the correspondence existing between these results and the insecticidal tests carried out upon these three specimens. The values obtained are undoubtedly affected by the fact that the method of extraction is not entirely selective. There are small and probably varying amounts of fats and fatty acids present, and it was impossible to free the roots entirely from attached particles of soil.

The amounts of non-toxic material precipitated by lead acetate can be estimated by extracting the ether-extracted powder with alcohol. Results are also shown in Table II.

SUMMARY AND CONCLUSIONS.

The toxic principles of *Derris elliptica* have been isolated and some of their more simple properties examined. A chemical method for evaluating the root has been outlined and a suitable extraction apparatus described.

1. The most important constituents of the root are a white crystalline derivative usually called "tubatoxin" and a resin or series of resins identical with the "derride" of Sillevoldt and the "tubain" of Wray. Besides these two, yellow crystalline derivatives and a liquid resin were isolated.

2. "Tubatoxin," the yellow crystalline derivatives and the resins contain methoxyl groups and these compounds appear to be inter-related. "Tubatoxin" by exposure to light, and by prolonged boiling with organic solvents is converted into three yellow crystalline products and a resin. This suggests that the "anhydroderride" of Sillevoldt may have been formed during the process of extraction and may not exist as such in the root.

3. The poisons from the root are readily extracted by means of organic solvents. 95 per cent. alcohol extracts them together with non-toxic derivatives. Benzene, dry ether, carbon tetrachloride are also good solvents for extraction purposes and have a selective dissolving action on the poisons. Petroleum derivatives are not suitable for complete extraction. Prolonged boiling with solvents may cause some loss of toxicity in the extracts owing to chemical change in the "tubatoxin." For economic purposes, benzene and its congeners or alcohol are probably the most suitable extraction reagents, provided the temperature of extraction is not allowed to rise too high.

4. The root may be evaluated by chemical means by extracting the dry root with dry ether and the genuineness of the extracts confirmed by the determination of the methoxyl content by the Zeisel method. Extracts from different deliveries varied between 7 and 22 per cent. and the content of CH_3O in the extracts between 13·5 and 14·7 per cent. A qualitative test for "tubatoxin" devised by Dr Durham is outlined on p. 5.

5. The amounts of the non-toxic constituents vary widely in different consignments. They seem to have some value as emulsifying and wetting agents. As the root however arrives in this country in a dry state in which the constituents have probably coalesced the use of foreign emulsifying and wetting reagents is necessary and for maximum efficiency the use of organic solvents for preparing highly dispersed suspensoids appears advisable.

LITERATURE CITED.

(1) NEWBOLD. *British Settlements in Malacca*, II, 399, 400, 406.
(1886). *The Pharmaceutical Journ. and Trans.* (3), XVII, 5.
(2) —— (1890). *The Pharmaceutical Journ. and Trans.* (3), XXI, 559.
(3) OXLEY (1848). *Journal E.I. Archipelago*, 646.
(1886). *The Pharmaceutical Journ. and Trans.* (3), XVII, 5.
(4) —— (1877). *Kew Gardens Report*, 43.
(5) —— (1886). *Pharmaceutical Journ. and Trans.* (3), XVII, 5.
(6) GRESHOFF (1890). *Pharm. Journ. and Trans.* (3), XXI, 559.
(7) WRAY, L. (1892). *Pharm. Journ. and Trans.* LII, 61.
(8) VAN SILLEVOLDT, H. E. TH. *Ned. Tijd. Pharm.* II, 246.
(1899). *Arch. Pharm.* CCXXXVII, 595.
(1900). *Journ. Chem. Soc.* A 1, 109.
(9) POWER, F. B. (1903). *Pharm. Arch.* VI, 1.
(10) CAMPBELL, J. A. *Journ. Straits Branch Royal Asiatic Soc.* LXXIII, 129.
(11) ISHIKAWA, T. (1917). *Chem. Absts.* 2370.
(1916). *Fr. Tokyo Igakkwai Zasshi*, XXX, 45.
(1917). *Jap. Med. Lit.* I, 7.
(12) McINDOO, N. E., SIEVERS, A. F., ABBOTT, W. S. (1919). *Journ. Agric. Research*, XVII, 177.
(13) PERKIN, W. H. (1903). "Simplification of Zeisel's Method of Methoxyl and Ethoxyl Determinations." *Journ. Chem. Soc. Trans.* LXXXIII, 1367.

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A QUANTITATIVE STUDY OF THE INSECTICIDAL PROPERTIES OF *DERRIS ELLIPTICA* (TUBA ROOT)

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(With 3 Text-figures.)

PROLOGUE.

THE work here described has its origin in a demand on the part of horticulturists for additional insecticides, a demand which during the war became more insistent owing to the high price and, often, scarcity of the few substances known to be of real use for spraying purposes. As originally planned, the investigation was to consist in a preliminary exploration of a wide range of possible substances, to be followed by a more detailed examination of those most likely to give practical success. This programme has been adhered to in the main, a number of substances including Yahoo Root, Mowrah Seed, seed of a Tephrosia, Serjania paucidentata, having been tested in a preliminary manner. Some departure from the original plan has, however, been made in regard to one substance, the root of the plant *Derris elliptica*, commercially known as Tuba Root, which has been investigated in somewhat greater detail than would have been called for in a mere preliminary survey. This departure was made partly because at the time when the work started a contact insecticide effective against caterpillars was specially needed and also because the earlier experiments indicated the desirability of developing methods on a substance available in relatively large quantities, which was the case in regard to *Derris*. The former reason naturally directed the choice of test insects towards caterpillars of various species, which have been retained throughout, and the point is mentioned to explain why poisons which appear to act essentially as contact insecticides have been tested chiefly on caterpillars and little on sucking insects such as aphides, against which contact insecticides are more usually needed.

With regard to the development of technique, the difficulties from the biological aspect have proved serious and justify some discussion (the chemical and physical problems are discussed on a later page).

In the first place it is clear that where the relative toxicity of a number of different substances has to be measured, the test insects ought in theory to be similar as regards species, age, health and environment; secondly, they should be available in very large numbers and this at all times of the year. It has not yet been possible to meet these conditions. The first series of experiments were carried out with caterpillars both bred from eggs and collected in the field, chiefly with those of the Cabbage White Butterfly (*Pieris brassicae*), the Lackey Moth (*Malacosma neustria*), the Buff-tip (*Phalera bucephala*), the Gooseberry Sawfly (*Pteronus ribesii*) and those of another Sawfly (*Phymatocera aterrima*). While experiments on these subjects were in progress attempts were also being made to find a species of which the caterpillars would (under artificial treatment) be available throughout the year.

Finally a Noctuid moth, *Hadena oleracea*, was chosen as a species likely to fulfil this condition. This common British insect, which under outdoor conditions is partially double-brooded, has recently established itself in tomato houses where it appears to breed continuously as long as the requisite heat is maintained. Owing to the kindness of Dr Lloyd (then of the Lea Valley Experiment Station) a large number of autumn pupae were obtained from the Lea Valley and were kept in an incubator at a temperature of 82° F., the atmosphere being practically saturated. Moths emerged freely after about six weeks and paired readily in cages 2 feet square kept in a greenhouse in which the temperature ranged from 60-70° F. Eggs were laid in abundance, hatched in about four weeks, and a large number of young larvae were thus obtained in winter and early spring. At this point difficulties arose, for although it proved possible to rear larvae in hundreds, the attempt to produce the thousands required was a complete failure owing to the high mortality when the larvae were about half grown. Contrary to expectation, difficulties increased with the advent of summer owing to the irregular behaviour of the pupae from the winter larvae, which only produced moths one or two at a time over a period running into many months. There were therefore seldom sufficient moths out at any one time to allow for fertile repairings. Comparatively few eggs were laid and the final result was a number of small broods of larvae, all of different ages and none large enough to be of use in testing.

The few moths obtained from these larvae showed evidence of decadence, laying a large proportion of misshapen and infertile ova, and although the strain is still in being it is not proving of much use for experimental purposes.

Similar results as regards irregularity in emergence have been recorded with tropical insects and it would seem likely that in most species a rest at some period is essential and that it is taken whatever may be the climatic conditions. When such conditions are uniformly favourable to the active stage of a species then the rest is taken quite irregularly, so that it is not possible to observe definite broods or generations.

Experiments with *H. oleracea* not proving successful, trials were concluded in so far as this paper is concerned with Lackey larvae and silkworms, which were reared from the egg in large numbers specially for the purpose.

The production of a suitable species in sufficient numbers was, however, only one difficulty; the methods of applying the insecticide to the test subject also gave considerable trouble. The first method used and the most simple was ultimately adopted for all the experiments here described, and consisted in dipping each caterpillar for a definite period (10 seconds) in the liquid to be tested. The caterpillars were then placed in jars containing the foodplant, and a few pieces of filter paper to absorb any excess fluid introduced with the wet caterpillars, the results being checked periodically during the subsequent 48 hours. Naturally the liquids varied greatly in their wetting power, while equally the different species of caterpillar were more or less difficult to wet. Further, a hairy caterpillar such as the Lackey or Buff-tip retained the fluid for a much longer time than a smooth caterpillar. It has not yet been possible to eliminate these disturbing factors, and all that could be done was to add a surface tension reducer which gave as thorough a wetting as possible, Saponin—to which the larvae showed no marked reaction—being usually used for the purpose.

Taking these various factors into consideration, great numerical accuracy cannot be claimed for the biological side of the work, but nevertheless it is believed that from the qualitative point of view the results are substantially correct. Quantitatively they may be regarded as of a fair order of accuracy.

In all cases great care has been taken to draw conclusions only from those experiments of which the results were undoubted, and even at the risk of losing matter of interest trials were discarded whenever there was a suspicion that a disturbing factor had been introduced—as, for instance, when deaths from disease or the attacks of parasites occurred in the main stock from which the trial subjects were selected. Deductions were based only on biological observations which were beyond dispute and may thus be regarded as fully justified.

Much yet remains to be done from the entomological standpoint, notably with regard to the physiological action of *Derris*, which would seem to involve problems of a nature not apparent in the case of such an insecticide as nicotine. The poisonous constituents of *Derris elliptica* are found to be relatively non-volatile, insoluble and solid. It might therefore be expected that their maximum efficiency can be secured only when special means have been taken to ensure that they are finely dispersed through the test fluid. On the other hand, from the physiological standpoint it is not altogether clear how non-volatile and insoluble solids function as contact insecticides, which seems to be the case. There has as yet been no opportunity for tackling either this or the other biological problems which have arisen in the course of the work, and a satisfactory conclusion to the whole investigation is to some extent dependent on their solution. It is felt, however, that the results already obtained should not be held over pending work on problems of a fundamental nature and in consequence this paper, which must be regarded as a "progress report," is published.

The entomological part of the research was carried out by Messrs Fryer and Stenton of the Plant Pathology Laboratory of the Ministry of Agriculture, the chemical side by Messrs Tattersfield and Roach of the Rothamsted Experimental Station. The insecticide tests were done conjointly.

We have pleasure in expressing our thanks to Mr Collin for much valuable preliminary entomological work.

Insecticide Tests.

Two different methods for estimating the insecticidal properties of the root and its various extracts were employed:

Method (1). A qualitative method carried out on a number of different insects and characterised by the comparatively small number of individuals used for each test. It consisted in grinding up the root, or the constituents of the root, with distilled water, dipping the insects for a period of 10 seconds into the resulting emulsion, mixture or filtrate, as the case might be, according to the point that required elucidation. In addition to this a few experiments were carried out in which soap and starch were incorporated with the mixture to act as a suspensory material. In this way information was obtained as to how the pure aqueous mixture or solution reacted physically with the insect, as, for instance, how it wetted, and whether the film remained permanent on the body or contracted into globules, and, again, how readily the various types of caterpillars were killed when brought into contact with the aqueous mixtures or solutions of the poisons.

The liquids used differed greatly in their wetting power and the several species of caterpillars, although varying somewhat, were on the whole difficult to wet. In the earlier experiments the aqueous mixtures of ground root were found to possess wetting properties to a moderate extent, which seemed however to decrease somewhat in accordance with the age of the root. The extracted constituents showed the property of wetting to only a small degree, thus probably affecting the results profoundly. As a rule hairy caterpillars such as the Lackey and Buff-tip wet more easily than others, but this is not invariable, as the relatively glabrous larvae of the gooseberry sawfly were not difficult to wet. The results are set forth in Table I.

The table is divided up into several groups, Group I refers to experiments carried out with finely ground root *Z*[1]. It is obvious that the root merely macerated with water is fairly poisonous to a number of insects at a 2 per cent. concentration, although still toxic at 1 per cent. and 0·5 per cent. it is less so than at the higher concentrations, and an addition of soft soap does not greatly help matters. Autoclaving the root to a temperature of 120° C. slightly lessens its toxic properties. That the poison is not volatile to any great extent is evidenced by the fact that the distillate in steam is not toxic. Exp. 8 was carried out to see whether aqueous mixtures would lose their toxic properties on standing, and by allowing fungi and bacteria to grow freely in the liquid. This mixture stood for one month; the resulting material seemed less toxic to *P. brassicae*, but on the whole the diminution was not great. The extraction experiments in Group II were carried out, to find out the most convenient way of isolating the poisons. It is obvious that the organic solvents, particularly alcohol, do extract the poisonous material and leave an inert residue behind. Group IV indicates the toxic properties of the pure poisons isolated from the root. The fact emerges that these pure substances and extracts do not appear as poisonous as the emulsions ensuing from maceration of the root with water. In the case of the extracts this is readily explained by the fact that prolonged drying at 100° C. causes chemical change in some of the toxic constituents. The

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[1] Three commercial samples of Derris Root labelled *X*, *Y*, *Z* for convenience were used in these experiments. Their derivation will be found on p. 13.

Table I.

	Material used and Description of Test	Insects used					
		M Neustria	H padellus	P. brassicae	N ribesii	P. aterrima	P bucephalae
	Ground root experiments (Group I)						
1.	2 % ground root macerated with water	×	×	×	×	.	×
2.	1 % „ „ „	.	.	×	×	.	.
3.	2 % air dried root (ground). Made up 20 hours and filtered	.	.	*	×	.	.
4.	2 % ground root, autoclaved to temp. 120°. Made up 20 hours and filtered	.	.	+	.	+	.
5.	0·5 % ground root. Made up 20 hours and filtered	.	.	*	*	*	.
6.	Ground root distilled in steam. Distillate	.	.	O	.	.	.
7.	„ „ „ Residue	.	.	– O	×	.	.
8.	2 % ground root aqueous extract, allowed to ferment	.	.	– –	×	×	.
9.	1 % ground root + ·5 % soft soap (*vide* No. 2)	O	.	.	.	.	.
10.	1 % ground root + ·8 % starch	O	.	.	.	.	.
11.	0·5 % soft soap	O	.	.	.	.	.
	Extraction experiments (Group II)						
12.	2 % ground root No. 1 above (for comparison)	×	×	×	×	.	×
13.	1 % „ „ No. 2 „ „ „	.	.	×	×	.	.
14.	2 % of the alcoholic extract of ground root (free from alcohol)	+	.	.	.	.	.
15.	1 % „ „ „ „	O	O	.	.	.	.
16.	Alcoholic extract (free from alcohol) later experiment	.	.	×	×	×	.
17.	Residue from alcoholic extraction in No. 14	O	.	.	.	.	.
18.	2 % of carbon tetrachloride (CCl_4) extract	.	.	O	× ×	.	×
19.	Alcohol extract of residue of 18	.	.	–	O *	.	*
20.	Alcohol extract of ground root. No. 16 above	.	.	×	×	×	.
21.	Pentane extract of alcoholic extract No. 20	.	.	.	×	×	.
	Saponification experiments (Group III)						
22.	Alcoholic extract before saponification. No. 16 above	.	.	×	×	×	.
23.	„ „ after saponification with KOH	.	.	–	+	+	.
24.	Unsaponifiable fraction of 23	.	.	*	*	*	.
25.	Saponifiable fraction of 23	.	.	.	O	.	O
	Experiments with derivatives from root (Group IV)						
26.	0·5 % yellow crystals. Mixture of tubatoxin and anhydroderride	.	.	.	×O	.	×
27.	0·5 % tubatoxin	.	.	.	×	.	+
28.	0·5 % anhydroderride	.	.	.	O	.	O
29 *a*.	Derride. Resin from root. Not filtered	×	.	.	.	.	.
29 *b*.	„ „ „ Filtrate from *a*	×	.	.	.	.	.
30.	Oil (liquid resin) contains tubatoxin	.	.	.	.	.	×

The symbols employed in this table have the following significance:

× = 100 per cent. deaths.
+ = Severe sickness clearly due to experiments, often with deaths.
* = Sickness definitely due to experiments.
O = No effect.
– = Uncertain—no conclusions permissible.

case of the pure substances and their apparently lower toxicity is discussed below. The saponification experiments were carried out with the object of seeing whether this would result in loss of toxicity—it does so. It was considered possible that some slight reaction of this type might ensue upon mixing this root with alkalies or soaps. There is, however, little danger of this taking place at ordinary temperatures, particularly if the powdered root mixture is fairly dry.

Method (2). The main fact arising from Table I is that when tested by method No. 1 the pure products (Gp IV) derived from the roots do not appear so toxic as the emulsions (Gp I) prepared from the fresh root by mere maceration with water. This result may arise from a variety of causes.

1. There may be constituents in the root which maintain the poisons in a very fine suspensoid condition.
2. These emulsifying constituents may enable the mixture to wet and to spread over the body of the insect.
3. The constituents of the root may have undergone some chemical change in the process of extraction.

Points 1 and 2 are of considerable importance, but in the absence of precise physiological knowledge as to how Derris poisons affect insects they cannot be usefully discussed. It can only be pointed out that in whatever way the poisons obtain entry to the insect, the size of the particles present in the fluid and the wetting power must have a direct bearing on the efficacy of the poison as an insecticide.

Point No. 3 could be determined only by the elaboration of a more exact quantitative method for testing the root and its products. This involved a considerable amount of work on methods of suspending in water the solid poisons in as fine a condition as possible, in such a way that the materials from the root were the only effective toxic constituents present and any variation in that toxicity resulted from a change brought about in the physical condition of the poisons.

The following method for preparing finely suspended mixtures of poisons was adopted: an alcohol extraction of the root made in the cold was slowly poured into a solution of saponin in water, and the mixture freed from alcohol by distilling off under fairly high vacuum at a temperature not exceeding 35° C. For convenience the concentrations of these suspensoids are expressed in terms of equivalent root—that is to say, they contain as much poison as could be found in mixtures containing the stated percentage of root.

(The mixture froths somewhat when the alcohol is reduced below a critical amount, but, with care, loss due to this need only be slight.)

The pure poisons were dissolved in a minimum quantity of alcohol and treated in the same way, a rather larger amount of saponin being necessary to render the tubatoxin suspensoid permanent. In this manner suspensoids of known strength were prepared, and by the addition of saponin solutions of appropriate concentrations a whole series of mixtures of constant saponin content but varying progressively with respect to the amount of poison present could be readily made. It was our intention to use these mixtures as a spray, but as suitable apparatus was not available, a dipping method was again employed.

A known number of insects, usually five or ten, were immersed for ten seconds in each of a series of known dilutions, and then placed upon their normal food supply and kept under observation for a period of two to three days.

Table II.

Insect used: larvae of Tomato moth (*Hadena oleracea*). No. of insects per test—5. Dipped for 10 seconds. 1 % saponin used for *X* and *Z*, except those figures in brackets which refer to ·2 % saponin.

	Root *X* Ether extract = 21·8 %			Root *Y* Ether extract = 17·6 %			Root *Z* Ether extract = 7·1 %		
% of root	No. used	No. killed	No. partially affected	No. used	No. killed	No. partially affected	No. used	No. killed	No. partially affected
2·0	5	4	0	—	—	—	5	0	0
3·0	5 + (5)	3 (2)	0	—	—	—	5	0	0
3·5	(5)	(4)	(1)	—	—	—	—	—	—
4·0	5 + (5)	5 (4)	(1)	(5)	(4)	(1)	5	0	1
6·0	5	5	0	(5)	(3)	0	5	3	0
8·0	—	—	—	(5)	(4)	(1)	—	—	—
9·0	—	—	—	—	—	—	5	3	0
10·0	—	—	—	(5)	(4)	0	(5)	(1)	(1)
12·0	—	—	—	—	—	—	(5)	(1)	(1)
14·0	—	—	—	—	—	—	(5)	(1)	0
16·0	—	—	—	—	—	—	(5)	(1)	0

Dipping for 10 seconds in distilled water and 1 % solution of saponin was without effect.

Table II shows results obtained by dipping larvae of *Hadena oleracea* in suspensoids of the alcoholic extracts from roots *X*, *Y*, *Z*. The mean percentage of ether extract[1] is also included for purposes of comparison. The wetting was found to be good. Unfortunately, as only a limited number of these larvae were available, the results are not complete and we were unable to test out upon them the various pure constituents of the root. Controls with 1 per cent. saponin, a percentage never exceeded, and water alone were tested at the same time and proved to have no effect on the larvae.

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[1] See p. 13 for method proposed for evaluating root.

Inspection of this table indicates that there is a difference in the toxic properties of the various consignments of this root. The results, although not complete enough to enable us to draw final conclusions, are in general agreement with those obtained in a similar manner using silkworms (see Table III). In the case of root *Z* it was found that on increasing the concentration beyond 9 per cent. a very imperfect suspensoid was obtained. This probably accounts for an actual decline in the proportion of deaths when these higher strengths were tested. Irregularities in the data show that sufficient numbers of insects have not been taken to eliminate the random variations in individual resistance.

Table III.

Toxicities to silkworms of different consignments of tuba root.

Ether extract of root $X = 21{\cdot}8$ %. Root $Y = 17{\cdot}6$ %. Root $Z = 7{\cdot}1$ %. 1 % saponin.
Insect used—silkworm. No. per test—10. Dipped five at a time for 10 seconds.

Description of test	Root %		% of dry ether extract	No. dead	No. moribund	No. slightly affected	Total no. affected	Marks awarded
Root *X*	2·0	equivalent to	0·436	10	0	0	10	100
	1·0	,,	0·218	10	0	0	10	100
	0·5	,,	0·109	10	0	0	10	100
	0·25	,,	0·0545	10	0	0	10	100
	0·125	,,	0·0272	7	0	0	7	70
	0·025	,,	0·00545	1	2	0	3	20
	0·0125	,,	0·0027	1	0	0	1	10
Root *Y*	2·0	,,	0·352	10	0	0	10	100
	1·0	,,	0·176	10	0	0	10	100
	0·5	,,	0·088	10	0	0	10	100
	0·25	,,	0·044	8	0	0	8	80
	0·125	,,	0·022	6	0	0	6	60
	0·05	,,	0·0088	1	1	0	2	15
	0·025	,,	0·0044	0	0	0	0	0
	0·0125	,,	0·0022	0	0	0	0	0
Root *Z*	20–2	,,	1·42–·142	10 each	0	0	10 each	100 each
	20–2	,,	1·42–·142	,,	0	0	,,	,,
	1	,,	·07	9	1	0	10	95
	0·5	,,	·035	6	0	4	10	70
	0·25	,,	·0175	4	1	3	8	52·5
	0·1	,,	·00875	0	0	0	0	0
Saponin solution (aqueous) 10 %			—	2	0	0	2	—
Ditto 5, 2, 1, 0·2 %			—	0	0	0	0	—
1 % saponin and alcohol 10 %			—	1	0	0	1	—
,, ,, ,, ,, 5			—	0	0	0	0	—
,, ,, ,, ,, 1			—	0	0	0	0	—
,, ,, ,, ,, 0·5			—	0	0	0	0	—
,, ,, ,, ,, 0·1			—	1	0	0	1	—
Alcohol solution 10, 5, 1, 0·5, 0·1 %			—	0	0	0	0	—

As there were available a large number of silkworms (*Bombyx mori*) free from disease, of known history, of about the same age and reared under exactly the same circumstances throughout, they were employed on a series of tests. The mixtures were made up and the tests carried out in the same way as described, except that ten insects were usually dipped, five at a time. The results are shown in Table III, Column 1 being expressed as described on p. 24. As the limit of toxicity is approached these results tend to become somewhat uncertain owing to variation in insect resistance. Many more larvae than we had at our disposal would have been required for the application of exact statistical methods and therefore to enable us to plot curves showing quantitative effects, different values were given to those which were killed and those partially affected. "Marks" were therefore awarded as follows:

A death was allowed ten marks.

A *moribund* condition five marks.

An affected but not *moribund* condition 2·5 marks.

The results were added up, calculated to a basis of 10 where less than that number of larvae had been used, and the sums of marks plotted against the amount of root or poisons employed in preparing our mixtures.

An inspection of Table III and Diagram I show very clearly that the ether extraction method (see Tattersfield and Roach, *ibid.* p. 13) is sound for purposes of evaluation. They indicate that root *X* is slightly but not very greatly superior to *Y* and that both of them are considerably superior to *Z*. The 50 per cent. death points are the most suitable for comparison. In Diagram II the results are presented differently. The toxicities of the different samples are calculated to their content of poison as estimated by the dry ether extract and these are plotted instead of the gross percentage of root; on the same diagram are plotted the results obtained in the same way with a sample of commercial nicotine of 92 per cent. strength (calculated to 100 per cent.).

It will be seen from Diagram II that a line can be drawn passing approximately through the various points that indicate the toxicities at the different concentrations of the samples *X*, *Y*, *Z*. The toxicity points of sample *X* are on the whole to the left of the line while those of *Y* and *Z* are on the right. Samples *Y* and *Z* both contain much more non-toxic material precipitated by lead acetate than does *X*, and a little of this would be extracted along with the poisons, as is borne out by the fact that the amount of methoxyl contents (see Table II, p. 15) of *X*, *Y* and *Z* descends in that order from 14·7 to 13·5. The results obtained are very close considering the difficulties encountered

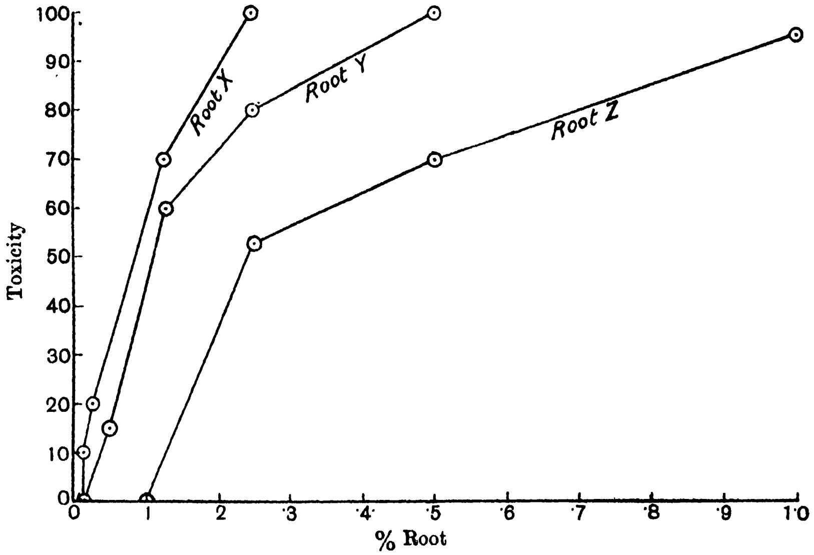

Diagram I. Showing toxicity to silkworms of different samples of tuba root.

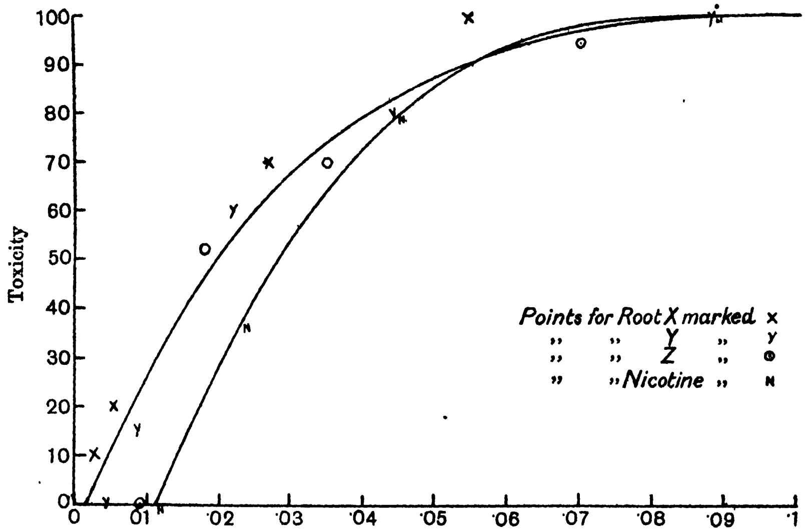

Diagram II. Showing relation between the toxicities of the various samples of root and the amount of ether extract.

in carrying out these insecticidal tests quantitatively. The diagram shows the extracts to be slightly but definitely more toxic than nicotine.

Table IV and Diagram III show the toxic relationships of the various pure products isolated from the root.

Table IV.

Toxicities to silkworms of products derived from tuba root and of nicotine.

No. used per test = 10 dipped for 10 seconds, 5 at a time. 1 % saponin.

Description of test	% poison	No. dead	No. moribund	No. slightly affected	Total No. affected	Marks awarded
Tubatoxin Finely dispersed	0·1	10	0	0	10	100
	0·05	10	0	0	10	100
	0·025	8	0	2	10	85
	0·0125	3	0	3	6	37·5
	0·005	3	0	2	5	35
	0·0025	3	0	0	3	30
	0·00125	1	0	0	1	10
	0·0005	0	0	1	1	2·5
	0·00025	0	0	0	0	0
Resin *A*, fraction 2, M.P. 72–82° C. Precipitated by pentane from ether solution	0·5	10	0	0	10	100
	0·2	10	0	0	10	100
	0·1	10	0	0	10	100
	0·05	6	1	0	7	65
	0·0125	1	1	0	2	15
	0·005	1	0	0	1	10
	0·0025	0	0	0	0	0
Resin *A*, fraction 3, M.P. 62–76° C. Precipitated by pentane from ether solution. Large excess of pentane	0·5–0·2	10	0	0	10	100
	0·1	10	0	0	10	100
	0·05	10	0	0	10	100
	0·025	8	2	0	10	90
	0·0125	6	0	4	10	70
	0·005	8	0	4	10	90
	0·0025	8	0	2	1	85
Resin *A*, fraction 4, M.P. 60–64° C. Soluble in pentane and ether, insoluble in pentane	0·5–0·2	10	0	0	10	100
	0·1	10	0	0	10	100
	0·05	10	0	0	10	100
	0·025	6	0	4	10	70
	0·0125	2	0	3	6	27·5
	0·005	0	0	1	1	2·5
	0·0025	0	0	0	0	0
Resin *B*. Different sample corresponding to a mixture of *A* 3 and *A* 4 more recently prepared, insoluble in a mixture of ether 1 vol., petroleum ether 2 vols.	0·5–0·2	10	0	0	10	100
	0·1	10	0	0	10	100
	0·05	9	0	1	10	92·5
	0·025	9	0	1	10	92·5
	0·0125	8	0	2	10	85
	0·005	4	0	1	5	42·5
	0·0025	2	0	0	2	20
	0·00125	0	0	0	0	0
	0·0005	2	0	0	2	20
Nicotine	0·092	10	0	0	10	100
	0·046	8	0	0	8	80
	0·023	3	1	1	5	37·5
	0·0115	0	0	0	0	0
	0·0046	1	0	0	1	10
	0·0023	0	0	0	0	0

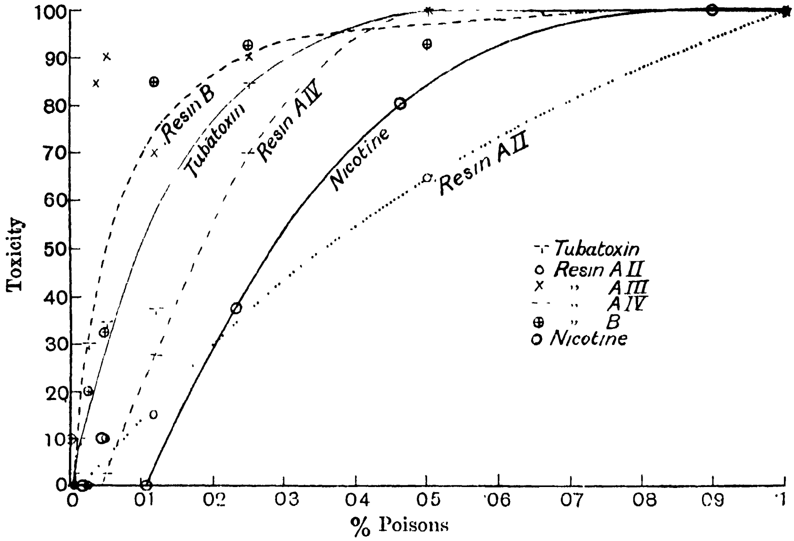

Diagram III. Shewing toxicities of constituents of tuba root in comparison with that of nicotine.

Points for Resin AIII are too irregular for a curve drawn through them to have meaning.

Table IV and Diagram III indicate that the pure products tubatoxin and the various fractions of the resin derride have a toxicity of an order comparable with that of nicotine. Details of fractions of derride *A* 2, 3 and 4 have been given on p. 12. Fraction *A* 1 was too small in quantity to be tested. Fraction 3 more closely corresponds to the resin discussed by Sillevoldt and Greshoff, than the others, while sample *B*, a resin

Table V.

Showing effect of filtering off suspensoid.

Insect used—silkworm. No. of insects per test—10.

Description of test	% of root	No. dead	No. moribund	No. slightly affected	Total No. affected	Marks awarded
Root *Z*	20–2	10	0	0	10	100
	1	9	1	0	10	95
	0·5	6	0	4	10	70
	0·25	4	1	3	8	52·5
	0·1	0	0	0	0	0
Filtrates from the above	from 20	7	3	0	10	85
	„ 16	6	3	1	10	77·5
	„ 12	8	1	1	10	87·5
	„ 8	8	1	0	9	85
	„ 4	1	0	2	3	15

prepared at a later date is analogous to a mixture of fractions 3 and 4. All these products were tested in a very finely suspended condition.

The effects of filtration on the toxicities of the suspensoids in water of the alcoholic extracts of one of the roots (*Z*) is shown in Table V.

Before filtration the suspensoid phase consists of particles ranging continuously from those of microscopic to those of molecular dimensions. An unknown quantity of particles of the sub-microscopic size would pass through the filter paper (No. 1 Whatman), but the total amount of the latter was relatively minute. After filtration the fluid still had a high toxicity. This fact seems to justify the belief that a more exact study of the relationship between fineness of dispersion and toxic action may lead to a more fundamental knowledge of the economic preparation of spray fluids. A tentative conclusion may be drawn from these facts, that for maximum efficiency the poisons of a spray fluid, when solid and non-volatile, must be present in as finely a divided suspensoid condition as possible. Early investigators noted that the fresh samples of root

Table VI.

Toxicities to silkworms of resins prepared from tubatoxin.

No. of insects per test—5. 1 % saponin.

Description of test	% poison	No. dead	No. moribund	No. slightly affected	Total No. affected	Marks awarded
Artificial resin No. 1 precipitated by pentane from solution in ether	0·5	5	0	0	5	100
	0·4	4	0	1	5	85
	0·3	5	0	0	5	100
	0·2	5	0	0	5	100
	0·1	3	0	0	3	60
	0·05	2	0	0	2	40
	0·025	0	0	0	0	0
	0·0125	1	0	0	1	20
	0·0005	1	0	0	1	20
	0·00025	0	0	0	0	0
	0·000125	1	0	0	1	20
Artificial resin No. 2 soluble in pentane and ether	0·5	5	0	0	5	100
	0·4	4	0	1	5	85
	0·3	5	0	0	5	100
	0·2	3	0	0	3	60
	0·1	1	0	0	1	20
	0·05	2	0	0	2	40
	0·025	1	0	2	3	30
	0·0125	0	0	0	0	0
	0·005	1	0	0	1	20
	0·0025	0	0	0	0	0
	0·00125	1	0	0	1	20

had higher toxic properties than those which had been long stored. Some indication of this was also observed in the present work, and the cause appears to be the drying up of the sap and the consequent coalescence of the poisons. Poisons in the milk-like product, resulting from the maceration of the fresh root are in a fine suspensoid condition, whereas with the aged root this is not the case and special means have to be adopted to achieve it.

Table VI shows the relationship between the toxicities of the resin produced by the prolonged boiling of tubatoxin with alcohol.

This reaction gives rise to two series of products:

(1) Crystalline derivatives (anhydroderride).

(2) Resin. This resin melts at 62–72° C. It can be fractionated to some extent by the precipitation of its ether solution by pentane.

The crystalline derivatives do not appear to have any toxic properties but unfortunately we did not have opportunity to test them in a finely divided condition. It was desirable to ascertain whether the resin derived from tubatoxin was poisonous or not, in order to discover whether any loss of toxicity was likely to arise from the action of boiling alcohol, if it were used for purposes of extraction.

If comparison is made with tubatoxin and resin *B* (derride), the artificial resin is distinctly less toxic than either. It appears therefore that prolonged boiling with solvents in process of extraction is not desirable.

Aphid Spray Trials.

Durham found aphides (*A. rumicis* and *E. lanigera*) had a considerable resistance to derris root and its products. McIndoo, Sievers and Abbott state that aphides sprayed with derris mixtures and extracts, behaved almost normally and showed no symptoms of paralysis, and that they died very slowly. Our experiments upon Bean Aphis tend to confirm these conclusions. Broad beans heavily infested with aphis were sprayed with emulsions of the root extract of strengths of 4, 2, 1, and 0·5 per cent. of the root. The toxic action was extremely slow and uncertain, while nicotine oleate in a dose containing ·05 per cent. nicotine was immediately and almost completely effective. This result on so vulnerable an insect is not easy to understand.

The wetting power of emulsions prepared from different consignments of root.

A few tests were carried out to ascertain if the non-toxic constituents of the root had any effect upon the wetting and spreading powers of its emulsions. Roots *X* and *Z* were tested. They were ground up with sand and water, made up to the required bulk in bottles, mechanically shaken for 20 hours, the coarsest particles allowed to settle and the turbid liquid decanted off for testing purposes.

Root *X* did not give a foam when shaken, *Z* however did, but it was not of a very permanent nature.

The tests were carried out as follows: five larvae of *Hadena oleracea* were dipped in different dilutions of these emulsions and a note made of the permanence of the film on the body. The results are shown in Table VII.

Table VII.

Effect of non-toxic materials on wetting properties.

Insect used—*Hadena oleracea.*

Test No.	Description	% of root present	% of non-toxic extractives present	% of toxic extractives present	Wetting properties, etc.
119	Root *X*	10	·41	2·2	Good—Continuous film
120	,,	5	·20	1·1	Film broke
121	,,	2	·08	·45	,, ,,
122	,,	1	·04	·22	,, ,,
123	Root *Z*	10	1·18	·71	Very good—Continuous film
124	,,	5	·59	·35	,, ,,
125	,,	2	·29	·17	,, ,,
126	,,	1	·14	·08	,, ,,

These results indicate that while toxic materials have little or no effects on the wetting and spreading powers of the extracts, some constituent of the non-toxic materials has, aqueous extracts of root *Z* showing distinctly better results in this respect than *X*. In none of the roots, however, were the inherent wetting and spreading properties sufficiently good to warrant reliance being put on them alone. For spraying purposes the addition of foreign wetting and spreading agents is essential if maximum efficiency is to be obtained, particularly when the dry powdered root is used.

SUMMARY AND CONCLUSIONS.

1. Extracts of *Derris elliptica* are shown to have a high insecticidal value, particularly for caterpillars. They are not so toxic to aphides.

2. The principles of the root toxic to insects are the white crystalline derivative, usually called "tubatoxin," and a resin of a golden yellow colour identical with the "derride" of Sillevoldt. Both these compounds when finely dispersed in water have considerable toxic properties to insects.

3. The dry root itself may be used in a finely powdered condition worked up with water together with soap or other emulsifying reagents.

4. As the pure poisons found in derris root are solids and only slightly soluble in water, their toxicity appears to depend upon their degree of dispersion.

5. A biological method of determining insecticidal properties quantitatively is described. It depends on dipping insects for a constant period of time in known strengths of highly dispersed suspensoids in dilute aqueous solutions of saponin. Results agreeing with those given by the chemical method already described (p. 13) were obtained. It enabled us to compare extracts of derris with nicotine. To certain caterpillars tubatoxin and derride are shown to be of the same order of toxicity as nicotine.

(*Received February 22nd*, 1922.)

BIOLOGICAL STUDIES OF *APHIS RUMICIS* LINN.

THE PENETRATION OF PLANT TISSUES AND THE SOURCE OF THE FOOD SUPPLY OF APHIDS[1]

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(With Plates I, II and 4 Text-figures.)

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I. INTRODUCTION.

THE food of aphids and the means by which it is obtained from the plant host, is an aspect of the biology of these insects which has been very little studied. Büsgen (6) was the first to seriously investigate the problem, more especially in relation to the production of "honey dew." Petri (15, 16, 17, 18) studied the question in the case of the vine *Phylloxera* and his researches have considerably advanced our knowledge of the subject.

In 1914 Zweigelt published his investigations on the subject and this paper, together with his later work on aphid galls in 1916, are important contributions to the literature.

Some smaller papers have been published but they are of a superficial nature and as they are, with few exceptions, referred to in the papers quoted above it is unnecessary to review them here.

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[1] A grant in aid of publication has been received for this communication.

The investigation is naturally largely a botanical one, demanding particularly a knowledge of plant cytology and vegetable physiology. The present writer therefore has attempted to describe actual observations, rather than to enter into a discussion of the numerous theories which naturally suggest themselves as an explanation of the problems which arise. It is felt that further research on the relationship existing between the host plant and aphids, especially as regards the physiological and chemical changes occurring in the plant, and the physiology of digestion in aphids, is necessary before a clear explanation of the problems can be established. An investigation of the subject is desirable in view of the increasing importance of aphids as possible vectors of certain widely spread diseases in plants.

My thanks are due to Dr A. D. Imms for his helpful criticism during the preparation of this paper.

II. TECHNIQUE AND METHODS.

The following aphids have been used in the investigations: *Aphis rumicis* L. on *Rumex, Vicia faba, Euonymus europaeus,* and *Chenopodium album. Macrosiphum rosarum* Walk. on rose trees and *Myzus cerasi* F. on peach.

Material was fixed with Farmer's fluid (absol. alc. 2 parts by volume and 1 part glacial acetic acid), or hot corrosive sublimate mixture (corros. subl. 3 gms., glacial acetic acid 3 gms., alcohol 60 per cent., 100 c.c.).

Small pieces of the infested plant were cut off quickly and allowed to fall direct into the fixative. In this way as many as 95 per cent. of the aphids were fixed with their stylets *in situ.*

Boiling water was useful for studying phloem contents. The infested part of the plant required was first bent over into 80 per cent. alcohol and immersed for a few seconds in order to stupify the aphids. It was then plunged into a large vessel of boiling water and the required parts quickly cut off *under* the water and allowed to fix. Air bubbles frequently enter the xylem vessels if the pieces are not cut off *under* the water and prevent proper fixation.

After fixation, the material was carried through in the usual way to the paraffin embedding bath. Great care must be exercised in handling the material in the various media or most of the aphids will fall off. Sections were usually cut 10μ thick, sometimes thicker for special preparations.

' The following stains were used:

Safranin and Delafield Haematoxylin.
Safranin Gentian-Violet orange G, triple stain.
Safranin and Lichtgrun (in clove oil).

The safranin formula used was, equal parts of saturated aqueous and saturated alcoholic solutions, the stain being allowed to act for 12 to 36 hours. Methylene blue, carbol fuchsin and ruthenium red were also used for certain preparations as indicated in the text.

Eau de Javelle and Schweitzer's reagent were used for certain purposes as well as other reagents which are referred to in the text.

III. THE MOUTH-PARTS AND THEIR RELATION TO THE PHENOMENON OF SUCTION.

The mouth-parts and mechanism of suction in aphids has previously been described by the writer(8). Awati(1) also described them in the Capsid bug *Lygus pabulinus* Linn., and recently Grove(11) has dealt with the same subject in *Psylla mali*. These authors have also reviewed the literature on the subject and the reader is referred to the above papers for detailed accounts.

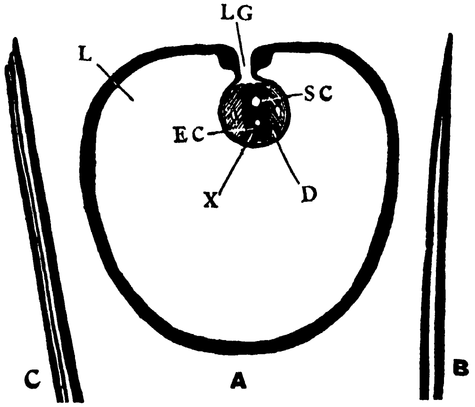

Fig. 1. A. Transverse section through the piercing organ of *A. rumicis* near the tip of the labium. The stylets composing the piercing organ are seen lying in the labial groove. L = labium; X = maxillary stylets; D = mandibles; LG = labial groove; SC = suction canal; EC = ejector salivary canal.

B. The pointed extremity of a mandible.

C. Side view of the extremity of the maxillary stylets showing the suction canal and ejector salivary canal. × 540.

Briefly, the parts associated with the mechanism of suction are the "forehead," which is composed of the clypeus and labrum, the labium or proboscis, a pair of mandibles and a pair of maxillary stylets.

In aphids the mandibles and maxillary stylets are pointed at their distal extremities (Text-fig. 1). In repose they lie closely apposed in the labial groove (*l.g.*) and function as one stylet, which I shall refer to in this paper as the piercing organ. It is this organ which penetrates into the plant tissues.

The internal or maxillary stylets (*x.*), on emerging from the head, become permanently closely apposed, thus forming two canals which lead to the extremity of the stylets (Text-fig. 1).

The dorsal and larger canal is the suction canal (*s.c.*) which carries the plant juices directly into the pharyngeal duct and so into the pharynx, from which they are forced back by the muscular action of the pharynx, along the oesophagus, into the stomach. The ventral one is the ejector salivary canal (*e.c.*). This canal is continuous with the efferent salivary duct and thus conveys the salivary secretion from the chamber of the salivary pump into the plant.

The mandibles lie close to the maxillary stylets, but sometimes separate from them when the piercing organ is withdrawn from the plant, and thus apparently three stylets may be seen.

A modification is necessary of my explanation in the paper referred to above, as to the manner in which the piercing organ is forced into the plant. The pincer-like action of the labium in assisting the penetration and withdrawal of the stylets in coccids, described by Berlese(2, p. 152; 3, p. 187) escaped my notice. This feature has been recently described in detail by Grove(11) in *Psylla mali.*

A similar action of the labium takes place in aphids, the penetration and retraction of the piercing organ being further assisted by the movements of the "forehead" and by the partial retraction and protraction of the labium.

When one examines *Aphis rumicis* killed *in situ* with the stylets inserted in the plant tissues, the labium is seen in many cases to be much shorter than normally. The basal portion is in fact withdrawn in a postero-ventral direction, somewhat like the inverted finger of a glove, a feature which the writer(7) has also described in *Eriosoma lanigera* Hausm. The labial groove at its distal end completely enfolds the piercing organ, thus holding it in position. The further the piercing organ is inserted into the plant, the further is the labium retracted. Usually however only about one half to two-thirds of the labium is withdrawn, but its posteriorly directed position, together with the forward movement of the freely articulating "forehead," allows the maximum amount of the piercing organ to be inserted into the plant.

I believe there is actually very little independent protraction or retraction of the maxillary stylets themselves, apart from the combined movements of the "forehead" and the protraction and retraction of the labium. Such independent movements would dislocate the delicate mechanism whereby the pharyngeal duct and efferent salivary duct fit respectively into the suction canal and ejector salivary canal (*vide* Davidson (8), Plate XXIV, figs. 1 and 9). The mandibles, on the other hand, are capable of independent retraction and protraction, which probably assists in the penetration of the tissues.

Aphis rumicis was observed feeding under the binocular microscope. When individuals were disturbed by touching them gently with a small brush, they usually made energetic efforts to draw out the piercing organ from the plant. The head was vigorously used in a tugging manner, and the labium became apparently longer. Eventually, when the stylets were withdrawn, they were seen to extend some distance beyond the tip of the labium, but the latter organ soon regained its normal length and automatically the stylets occupied the position of repose in the labial groove.

IV. THE PENETRATION OF THE PLANT TISSUES BY THE STYLETS.

The piercing organ resembles a flexible bristle and is readily bent in any direction. Its course in the plant tissues is associated with the search after nutritive cell sap, as is well illustrated by the richly branching stylet tracks found in the phloem. In cleared preparations of a piece of stem or leaf with aphids *in situ*, it can be seen passing through the epidermis into the deeper tissues. In serial sections however, it is only occasionally that a good length of the piercing organ can be seen in one section. This is due to its irregular course through the tissues, which results in only small pieces being obtained in the sections. Frequently, these small pieces of chitin tear the section and drop out. Plate I, figs. 1 and 2 and Plate II, fig. 4 show the piercing organ in the plant tissues.

Fortunately it is easy to demonstrate the course taken by the piercing organ because, when this structure is in the plant tissues, a tube-like sheath, the stylet sheath, forms round it (Plate I, fig. 1). The contents of the latter, when the piercing organ is withdrawn, stains readily with certain stains, especially safranin, forming a definite track, the ***stylet track*** (*vide* Text-figs. 2, 3, and 4). ✓

Sometimes the stylet track follows an irregular course in comparison with that of the actual piercing organ. This is doubtless due to three causes. (1) The dissolving action of the saliva. (2) The changing turgidity of the plant cells, which when the stylets are withdrawn squeezes the tract irregularly. (3) The action of the fixative which often causes shrinkage of the tissues, owing to the comparatively large pieces which have to be fixed.

Plate II, fig. 3 shows the direct course taken by the piercing organ.

When a piece of a heavily infested plant is fixed, it follows that many of the stylets fall out during the subsequent technique. There are therefore numerous stylet tracks which may not show in the sections, owing to the fact that they are empty and do not stain.

Furthermore, owing to the chemical changes which take place as a result of the penetration of the tissues, the action of the saliva and the action of oxidases, some stylet tracks stain faintly or not at all.

The filling of the stylet sheath, after the piercing organ is withdrawn, is doubtless produced by a welling up of the plant juices owing to the osmotic pressure in the tissues[1]. It is seen in Plate I, fig. 3, for example, that the contents of the stylet sheath have been forced out on to the outer surface of the epidermis. The nature of the stylet sheath will be considered more fully in a later paragraph.

With *Aphis rumicis* the piercing organ enters between the epidermal cells **and** usually follows an intercellular course in the cortex, although occasionally individual cells may be pierced through (*vide* Plate I, fig. 3; Plate II, fig. 2 and Text-fig. 3).

In young meristematic tissue and in tissues where intercellular spaces are absent, the penetration may be intracellular, although the course is usually along the line of the cell walls. *Vide* Text-figs. 2 and 3.

The intercellular course in the loose cortical tissue, clearly shown in Plate II, fig. 2 is I think the line of least resistance for the piercing organ. The resistent turgid cells would tend to deflect this delicate structure while, on the other hand, when passing between the cells, there is considerable lateral support for it and its fine needle point makes penetration comparatively easy. Furthermore, it appears clear that the salivary secretion is able to digest certain cell walls, more especially the middle lamella, thus greatly assisting the penetration process.

In some cases, especially when the plant is heavily infested, one can see the cells are forced apart by the passage of the stylets, and in others, they are torn and destroyed by the combined physical action of the stylets and the effect of the saliva. The effects of the stylets and of the salivary secretion on the cells will be discussed in a later paragraph.

With regard to the passage of the piercing organ between the cells, the high pressure exerted by the latter under osmotic influence or turgor, must be considered. It will be seen however (Plate I, fig. 3) that the saliva coming in contact with the cell contents, causes extensive plasmolysis of the cells, thus reducing the pressure. Even where extensive plasmolysis does not take place, the osmotic pressure of the cell is I think not a serious difficulty, owing to the delicate nature of the piercing organ and the action of the saliva. The importance of the passage of the piercing organ along the line of the cell walls is, that support is thus afforded it during penetration of the tissues.

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[1] See footnote on page 42.

Büsgen (6) classified the course taken by the stylets in plant tissues, into three distinct types. (1) A course leading to the vascular bundles, passing intercellularly through the cortex. (2) A course passing intracellularly through the cortex and not going to the vascular bundles. (3) A course leading to the vascular bundles passing intracellularly through the cortex.

It is difficult to sharply demarcate these three types of stylet tracks. Certainly types 1 and 3 may be classed together as one type. With *Aphis rumicis* the first is the usual type met with.

Type 2 on the other hand does occur in some cases. Petri, having shown that *Phylloxera vastatrix* on the vine may not reach the vascular bundles, contenting itself with the cortical cells, through which the piercing organ passes intracellularly.

Zweigelt (26) considers that the intercellular passage of the stylets is associated with an extensive intercellular suction, which is the principal method by means of which the cell sap is obtained.

My observations lead me to the view that in the cases investigated, intracellular suction is the most important method of obtaining nourishment, and that the significance of the intercellular passage of the piercing organ through the cortical tissues, is associated with easier penetration on its way to the vascular bundles. Although there is frequently a marked plasmolysis of the cells bordering intercellular spaces in which saliva has been secreted, this is not always the case, and only occurs when the cell wall is so affected that the saliva comes in contact with the cell contents. On the other hand, most sections show stylet tracks ending in individual cells.

The intercellular spaces contain chiefly water and dissolved gases. In the case of intercellular suction, the cell membrane would exert a selective influence on the passage of nutrient substances from the cell into the intercellular spaces. Of course in cases where the protoplasm of the cell is killed by the action of the saliva, its selective power would be destroyed and the case would be almost equivalent to a case of intracellular suction. This does sometimes happen, but often only a small quantity of saliva is found in the intercellular spaces, *vide* Plate II, fig. 2. The nature and occurrence of these irregular masses of saliva in the intercellular spaces, suggests that they are sometimes formed after the piercing organ has been withdrawn, by the pressure of the cells forcing the fluid along the stylet track, from between the cells.

One frequently sees a group of cells affected by the saliva, for example in the cortex of rose stems and in the phloem of peach tissue attacked by aphids (*vide* Plate II, fig. 1).

It is seen from the foregoing account, that with *Aphis rumicis*, the piercing organ follows a clean course (usually intercellular in the cortex, but occasionally passing through individual cells) to the vascular bundles, from the phloem elements of which it chiefly derives its nourishment.

V. THE STYLET SHEATH.

As the piercing organ penetrates into the tissues, the insect pumps saliva into the plant and the stylets thus become surrounded with plant juices and saliva. Owing to the action of the saliva, a precipitation or coagulation of substances results, which forms round the piercing organ a thin walled tube or sheath, the stylet sheath, Plate I, fig. 1, Plate II, fig. 4, which becomes irregularly thickened by a deposition of tannin[1]. When the piercing organ is withdrawn, it leaves a track in the tissues as explained on p. 39, which when stained with safranin, shows in sections as a definite reddish track, the stylet track.

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[1] The primary wall of the stylet sheath is probably of a cellulose nature, but as can be seen in Plate I, fig. 1 there is an irregular deposition of substances on its outer surface. Petri, 1908, p. 28 and 1911, p. 58 considers that the sheath is composed in *Phylloxera* of callose and insoluble calcium pectate, tannin being deposited some time after the actual wall of the sheath is formed. This author considers that the contents of the sheath is largely saliva, but it seems to the writer that watery solutions of a non-colloidal nature pass through the wall of the sheath and thus mixing with the saliva give the well defined stylet track as explained in the text. It is probable that the brown coloration often seen in the cells bordering the older stylet tracks is due to oxidase action.

The presence of tannin along the stylet track is considered by some authors as representing an attempt on the part of the plant to resist the attack of the insect. Thus in the case of aphids on rose trees, there is frequently a callose thickening of the walls of the cells attacked, being a reaction on the part of the cell, to the influence of the saliva. The nature of the ferment action of the saliva needs further study. According to some authors its action results in a large accumulation of soluble carbohydrates in the areas affected by the saliva. Tannins may probably be formed in the presence of excess of soluble carbohydrates. Naturally owing to the state of our knowledge these views are largely theoretical.

Sections of bean stem were tested for soluble carbohydrates by treating them with a few drops of 10 per cent. solution of thymol in 50 per cent. alcohol, followed by a few drops of concentrated H_2SO_4. The stylet sheath showed as a delicate, colourless tube passing intercellularly through the cortex. When treated with iodine in K.I., followed by concentrated H_2SO_4, the stylet track showed brownish yellow in contrast to the blue cellulose reaction of the cell walls. Eau de Javelle destroys the stylet sheath. When treated with Schweizer's cupra-ammonia reagent and stained with carbol fuchsin, the stylet track shows clearly passing along the middle lamella. This is especially clear when the track passes through the walls of the thickened cortical cells situated at the four angles of the stem in *Vicia faba.* Sections treated for 24 hours with 22 per cent. HCl in 95 per cent. alcohol and afterwards stained with aqueous methylene blue, show the stylet track very distinctly in relation to the cells.

The staining action of methylene blue, following treatment with HCl as indicated above, suggests the presence of pectic substances. Petri's researches on *Phylloxera vestatrix* referred to in the footnote on p. 42, shows that the sheath is due to a precipitation of substances formed by the reaction of the saliva on the cells, and consists of callose and insoluble calcium pectate, having on its outer surface a deposition of tannin.

It is evident that a generalisation as to the chemical composition of the stylet sheath in aphids cannot be made. It is probably dependent on the species of aphis and plant concerned. It may for instance differ in the case of gall forming and non-gall forming aphids. In this respect it is interesting to note that Petri was unable to stain the stylet sheath in the case of *Mytilaspis fulva* Targ. on the olive.

Büsgen considered that the stylet sheath acts as a supporting structure for the stylets. This function must be a relatively unimportant one, owing to its delicacy compared with the piercing organ. Furthermore, the stylets are well provided with strengthening grooves and ridges on their internal faces.

In some cases the stylet track may be seen completely investing a cell. This does not mean that the piercing organ has curved round the cell, but indicates the dissolving action of the saliva, following the middle lamella of the cell wall.

The stylet track does not always stain. This probably depends upon the chemical changes which may go on after the piercing organ is withdrawn.

VI. THE EFFECTS OF THE STYLETS AND SALIVARY SECRETION.

Owing to the needle-like extremities of the mandibles, the physical damage to the cells is not so extensive with aphids as with some other Rhynchota. For example in Capsid bugs the mandibles are serrated at the tip and considerable laceration of the tissues results. With *Macrosiphum* on the rose and *Myzus* on the peach, the cortical cells are often torn. In the case of *Aphis rumicis* on the bean however, the piercing organ takes a clean course through the tissues, and although plasmolysis of the cells occurs, there is very little tearing of the cells.

The salivary secretion exerts a diastatic action on the insoluble carbohydrates of the plant, changing them into soluble sugars; but in this respect the influence of diastase present in the plant itself, on the carbohydrates in the cell sap round the apex of the piercing organ, must be considered.

The salivary glands of *Aphis rumicis* were placed on an agar plate impregnated with starch, and after a time the plate was treated with iodine. In three cases, the areas where the salivary glands had been placed, showed white against the otherwise blue background of the plate, owing to the conversion of the starch by the action of the saliva. The diastatic action of the saliva is well established in Rhynchota in general. Petri has shown with *Phylloxera* that starch is converted into sugar in the affected cells, and similarly Zweigelt showed with certain aphids that there is an increase in sugars along the stylet track. It is of interest to notice that the starch grains (*G*) in Plate II, fig. 4 near the piercing organ, are smaller than those lying further away. This is one of the most important functions of the saliva, but the fact that saliva is frequently secreted, immediately the piecing organ enters the epidermis, suggests it is also important in assisting the penetration of that structure, by digesting the cell walls[1]. The remains of the salivary secretion is seen in Plate I, fig. 2 (coloured red), lying in the intercellular spaces bordering the piercing organ, near to the epidermis.

It would seem therefore, that the presence of a thick cuticle on certain plants may be of importance in preventing newly hatched aphids from piercing through to the underlying tissues. Cutin is notoriously resistent to the action of ferments and the initial piercing of the cuticle is probably a physical process. Older and stronger aphids might pierce the cuticle, but the presence of a thick cuticle suggests that the plant in general would not be attacked, although colonies of aphids might develop on the young growing shoots.

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[1] The cell wall is composed of cellulose in which a number of organic compounds may be deposited, forming the so-called hemi-celluloses, of which pectic substances are common.

When the saliva comes in contact with the cell contents, there is a marked plasmolysis of the cell and usually a migration of the protoplasm towards the source of the irritation, Plate I, fig. 3. The contents of the cell become disorganised, the nucleus swells considerably and eventually becomes a deeply staining, irregularly shaped, homogeneous mass. The cytoplasm becomes disintegrated and in extreme cases only a small layer of cytoplasm is left in the cell. The cells which are attacked eventually go brownish in colour presumably owing to oxidase action.

Even the lignified xylem vessels are acted upon by the saliva, the penetration of which is seen in Plate I, fig. 4.

The extent to which the local area attacked influences the surrounding tissues by a general distribution of the irritating salivary secretion is an important question. With *Aphis rumicis* there does not appear to be any spreading. It is more serious however in the case of *Myzus* on peach where many of the cells in the course of the stylet track are frequently torn and destroyed, Plate II, fig. 1.

Zweigelt found with *Aphis grossulariae* on *Ribes aureum*, that the cells were affected three or four rows away from the actual stylet track, and there was marked plasmolysis.

The spreading of the damage from the actual area penetrated, is seen more readily in some other Rhynchota, as for example in *Tomaspis saccharina* Dist. on the sugar cane, *vide* Williams (22).

Although the actual piercing through of the plant cells will eventually result in the death of those cells, the important factor is undoubtedly the action of the saliva. Smith (20) has demonstrated by experiment the harmful effects on plant tissues, of the saliva of certain Capsid bugs.

In sections of aphid-infested rose stems and also peach stems, one sometimes finds an irregular thickening of the walls of the cells in a badly infected area, due to the reaction of the cell under the influence of the saliva. Petri observed similarly with *Phylloxera* on the vine, a callose-like thickening of the walls of some of the affected cells. Zweigelt also observed a cellulose thickening of cortical cells in the rose stem. He also records that a similar feature, owing to the action of fungal hyphae (*Ustilago maydis*) has been observed by Guttenberg.

From the foregoing account, it will be readily seen that the plant exhibits many pathological features as a result of aphid attack, particularly owing to the influence of the salivary secretion. However it must not be overlooked that once the physiological relationships existing inside the cell are disturbed, owing to the influence of the saliva, the complex chemical changes which may result, cannot at the present stage of our knowledge be clearly understood. Further research is necessary on the action of the saliva of different species of aphids on different plant hosts and the changes which occur in plant tissues infected with salivary secretion. Further, the physiology of digestion in aphids requires to be investigated.

VII. Sources of the Food Supply.

The food of aphids is the cell sap of plants derived from various cells of the plant tissues, especially the vascular bundles. The numerous branching tracks so frequently seen in the phloem region, indicate that this tissue is particularly sought after. Text-fig. 2 shows a simple case of branching in the phloem of a young bean stem. However, cells of the epidermis, cortex, pith, mesophyll of the leaf and xylem vessels may be tapped for nourishment, especially when the host is heavily infested or in poor condition. In Text-fig. 3, which is a section near the tip of a young bean stem, the stylet track after passing through the cortex is branching out in the interfascicular or medullary ray tissue, two branches passing to the phloem and the other two to the pith cells. Plate II, fig. 3 shows a single track direct to the phloem and about to enter a companion

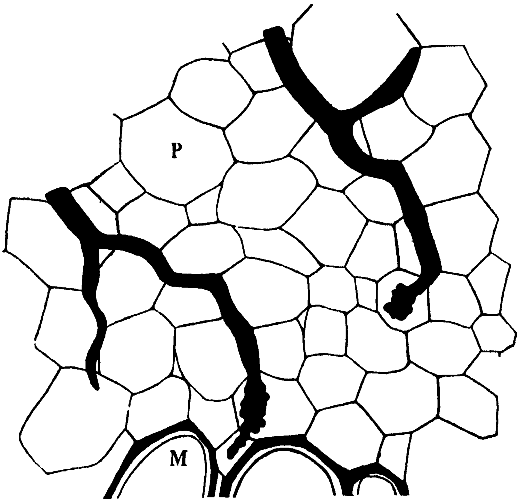

Fig. 2. Transverse section of a young bean stem showing branching stylet tracks of *Aphis rumicis* in the phloem and the sucking out of the cell contents. P = phloem; M = xylem. x 540.

cell. This insect was apparently killed soon after the phloem was reached and hence branches of the track had not yet been formed. Plate I, fig. 1 shows the actual piercing organ passing through the phloem and entering a companion cell.

An interesting case is seen in the transverse section of *Rumex* flower stem infested with *Aphis rumicis*, Plate I, fig. 4. Three stylet tracks pass into the xylem vessels, into which a quantity of saliva has been secreted. Again in Fig. 2 on the same plate the actual piercing organ is seen forcing its way through the thick pericycle sheath of a *Rumex* stem into the phloem.

I have found epidermal cells filled with saliva and the contents of the cells destroyed, the stylet track eventually ending in the first or second row of cortical cells. This probably represents cases in which the aphids were killed before the piercing organ had penetrated deeper into the tissues.

Cortical cells are frequently found plasmolysed and sucked out. This was especially noted with *Macrosiphum* on the rose (Text-fig. 4 *a*) and *Myzus* on the peach. This sucking out may be produced by a deliberate piercing of the cell (Text-fig. 4 *a*) or by the destruction of the cell wall by intercellular penetration and secretion of saliva.

It is seen that the piercing organ frequently follows the line of the cell wall. Apart from the value of this in assisting penetration as explained in a previous paragraph, it is economical, in so far that a greater number of cells are tapped than if the line of penetration was always directly intracellular.

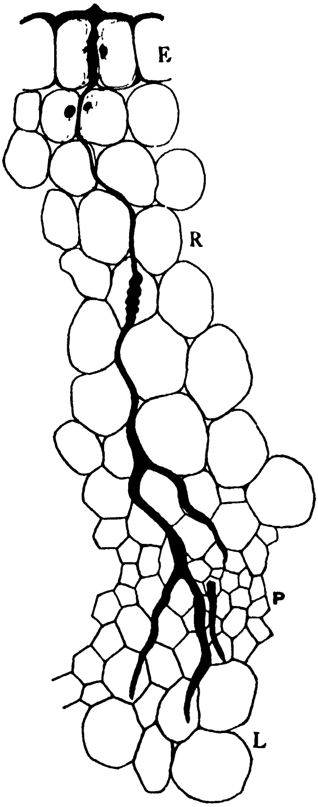

Fig. 3. Transverse section of a young bean stem showing branching of the stylet tracks of *Aphis rumicis*. The epidermis and two rows of cortical cells are shown in detail in Plate I, fig. 3. E = epidermis; R = cortex; P = phloem; L = pith. × 225.

Normally, *Aphis rumicis* lives quietly in close colonies on young parts of the plant or beneath the leaves. During a heavy infestation however, the turgor of the cells is considerably reduced owing to the action of the saliva and the withdrawal of cell sap by the aphids. The supply of sap therefore is greatly affected, a wilting of the shoot occurs and the insects in their search after food become restless, wander over the plant and tap the cells of many different tissues for nourishment. While normally the colonies below the leaves are situated on the mid rib or veins (the phloem being on the under side of the leaf), in heavy infestations they often wander over the leaf surface and puncture the spongy mesophyll tissue.

Two young bean plants were infested in a localised area, each with two apterous individuals of *Aphis rumicis.* After 24 hours this part of the plant was fixed with the aphids *in situ.* Only four stylet tracks were found in the sections and they passed to the phloem tissue.

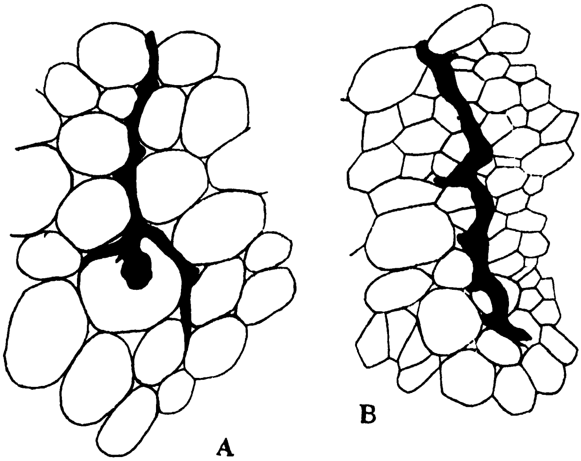

Fig. 4. Transverse section of stem of Rose showing:

A. intercellular passage of the stylet track of *Macrosiphum rosarum* in the cortex and sucking out of a cortical cell;

B. showing stylet track in the phloem. × 500.

Some species of aphids do, however, exhibit differences in their method of obtaining nourishment from the plant. Petri for example has shown that *Phylloxera* on the roots of the vine takes its nourishment from the cortical cells and does not reach the vascular bundles. It would appear that the differences in anatomical structure occurring in plants may affect the relative accessibility of the tissues rich in sap, especially the vascular bundles. The distribution of sclerenchyma, for example, may be important. Further there is the question of the differences which may occur in different species of aphids, as for example the length of the stylets. It must be remembered that it is the young forms which have to be especially considered.

The varying constitution and concentration of the cell sap of different plants and of the same plants at different periods of growth, is doubtless an important factor influencing the development of aphids. Speaking generally, the cell sap consists of a number of dissolved substances, which are the products of the metabolism of the plant, being usually acid owing to the presence of certain organic acids. The following substances may be present in cell sap (Strassburger's *Text-Book*, 1921).

(*a*) Inorganic salts, especially nitrates, sulphates and phosphates.

(*b*) Soluble carbohydrates, such as cane sugar, malt sugar, glucose and grape sugar.

(*c*) Amides, especially asparagin and albuminous substances. Alkaloids and glucosides are also often present. Tannin is frequently found in the cortical cells and some authors consider that on this account these cells are largely protected from aphid attacks. The presence of tannin associated with the stylet track has already been discussed.

From the anatomical and cytological considerations discussed in this paper, it is evident that the saliva can digest the celluloses of the cell wall, more especially the middle lamella which is largely composed of calcium pectate. Frequently one sees the stylet track passing intercellularly through the thick walls of the stout supporting tissue located at the angles of the stem in *Rumex* and *Vicia faba*. This tube-like track shows distinctly the dissolving action of the saliva.

The quality and consequent food value of the sap varies in the different tissues. The cell sap of the cortical parenchyma is moderately rich, but the phloem elements which serve to transport (sieve tubes) the rich products of assimilation throughout the plant, or as temporary storage cells (phloem parenchyma), have naturally a richer concentration of nutritive substances.

The intercellular spaces of the cortex are largely filled with water and dissolved gases. In the case of trees, the parenchyma cells of the cortex and pith become filled towards the end of summer with reserve substances, chiefly starch. When the buds burst in spring the action of the diastase ferments in the plant change these insoluble reserves into sugars, which are translocated to the buds.

Normally the supply of sap in the phloem is plentiful and it is rich in albuminoid substances, carbohydrates and inorganic salts, especially phosphates.

The xylem vessels (tracheae) are essentially vessels for conveying the watery solutions of organic and inorganic substances up the plant. Dixon and Atkins (9, 10) have shown however, that monosaccharides and disaccharides are also found in the sap of the tracheae in all the trees these authors examined, the concentration of these substances being greater in spring than in autumn.

We see therefore, that with *Aphis rumicis* the phloem elements of the vascular bundles, are the chief source of the food supply, although other cells of the plant may also be tapped for nourishment. The rapid development and reproduction of aphids on young growing tissue is evidently associated with the quality and rich concentration of the cell sap. These observations also indicate, that the varying susceptibility of different plants to aphid attacks, which the writer has recently investigated, is associated with the constitution and concentration of the cell sap. Investigation of the cell sap of plants at different periods of growth and under varied manurial and cultural conditions is necessary. While great stress has been laid on the value of soluble carbohydrates as food for aphids, one must not overlook the fact that proteids are equally important. The amount of proteid matter compared with carbohydrates in cell sap is relatively small. In young growing tissue on the other hand, the sap is rich in substances of the amino-acid type.

VIII. THE EXCRETION OF HONEY DEW.

Finally one may briefly refer to the honey dew of aphids, the production of which is closely associated with the digestive processes.

If with the aid of a lens, one watches *Aphis rumicis* feeding on a young succulent plant, it will be seen that the individuals frequently raise the posterior end of the abdomen and eject a large drop of liquid from the anus. This is the so-called honey dew, which often forms an adhesive layer on plants infested with aphids. In many cases this excretion becomes covered with a black fungoid growth, which grows rapidly on the medium. On *Euonymus europaeus*, *Aphis rumicis* excretes similar drops from the anus, but these dry and leave a white deposit on the leaves and the fungoid growth does not develop on them. This substance responds to the Fehling's solution test, indicating the presence of reducing sugars.

Büsgen records that fungi do not develop on the excrement of *Aphis padi* on Prunus nor of *Aphis euonymi* on *Euonymus*.

On some young *Euonymus europaeus* bushes which were heavily infected for experimental purposes, the writer observed the black fungus did develop on many leaves, but normally the fungoid growth does not develop on the excrement of *Aphis rumicis* on *Euonymus*.

The stomachs of apterous viviparous females fed on *Euonymus* and beans respectively, were examined five hours after feeding, on April 14th, in normal saline solution. In every case, of those fed upon *Euonymus*, the gut was found full of a white crystalline substance composed of long needle-shaped crystals. In the case of the aphids on beans, the stomach was smaller and filled with fluid and numerous oil globules, and there were only a few isolated crystals. Some a. v. ♀♀ from *Euonymus* were examined 30 hours after feeding and the large crystalline mass was still present in the stomach.

It would appear therefore, that the composition of the cell sap affects the nature of the excrement or honey dew. The medium thus produced may not in some cases be suitable for the growth of the fungus. Further the relationship between ants and certain species of aphids, the former being attracted by the honey dew, probably depends largely on the specific composition of that substance.

No recent analysis of the chemical constitution of honey dew has been carried out, but Boussingault (4) and later Brandes (5), showed that in the cases investigated there was a high percentage of sugars. Boussingault showed that there was an increase in dextrine and invert sugars, indicating a breaking up of the cane sugar of the plant juices by the digestive processes of the aphids. Aphids appear to feed almost continuously and large quantities of soluble carbohydrates are taken up in the cell sap. As these insects do not possess Malpighian tubules, the waste products, including surplus sugars, are passed out through the anus.

From the observations described above, it is clear that the cell sap is an important factor in relation to the physiology of aphids, a closer investigation of which, in relation to the composition of honey dew, may throw more light on the biology and physiology of these insects.

IX. SUMMARY.

(1) The food of aphids is the cell sap of plants, which they obtain by penetrating the tissues by means of a flexible, chitinous, piercing organ, which is composed of the maxillary stylets and the mandibles.

(2) The maxillary stylets are closely apposed, thus forming two canals, which extend to the extremity of the compound stylet thus formed. The dorsal canal is the suction canal along which the plant juices are conducted into the pharynx. The ventral canal is the ejector salivary canal down which the saliva is pumped into the plant.

(3) The penetration of the piercing organ is brought about by the retraction of the labium and the forward prolongation of the "forehead." The labium at its distal end grips the piercing organ in a pincer-like manner.

(4) With *Aphis rumicis* the piercing organ passes intercellularly through the cortex, only occasionally passing through individual cells. Eventually it reaches the vascular bundles.

(5) The saliva is able to dissolve a passage for the piercing organ through the walls of the cells. It is also able to convert starch into sugar.

(6) A well marked sheath, the stylet sheath, is formed round the piercing organ when it is in the plant tissues, its wall being composed of substances produced by the reaction of the saliva on the cell sap. It probably consists of callose and insoluble calcium pectate together with a deposition of tannin.

(7) The saliva causes plasmolysis of the cells and disorganisation of the cell contents.

(8) With *Aphis rumicis* on beans, the phloem elements of the vascular bundles are the chief source of the food supply, although other cells of the plant, such as the cortex and mesophyll of the leaf may be tapped for nourishment, especially when the plant is heavily infested. Frequently in *Rumex*, xylem vessels are tapped for food.

(9) The presence of a thick cuticle may prevent young aphids from piercing into the tissues and inhibit a general infestation of the plant.

(10) The sucking out process is usually intracellular, although intercellular suction sometimes occurs in the loose cortical tissue.

(11) In the case of *Macrosiphum* on the rose, occasional cells react under the influence of the saliva, resulting in a cellulose thickening of the cell wall of affected cells.

(12) The varying constitution and concentration of cell sap in different plants is an important consideration in respect to the biology and physiology of aphids.

(13) The more favourable development and reproduction of aphids on certain plants is probably associated with the nature of the cell sap, and an investigation of the cell sap of plants under varying cultural conditions and manurial treatment appears to be necessary.

(14) The composition of the excrement of aphids, or honey dew, depends upon the species of plant and aphid concerned, and is in close relationship with the composition of the cell sap of the plant and the digestive processes of the aphid.

X. REFERENCES.

(1) Awati, R. P. (1914). *Proc. Zool. Soc. London*, 685–733.
(2) Berlese, A. (1893). *Riv. Pat. Veg.* ii, 152.
(3) —— (1895). *Ibid.* v, 18.
(4) Boussingault (1872). *Compt. Rend.* lxxiv, 87.
(5) Brandes, G. (1893). *Zeitschr. f. Naturwiss.* lxvi, 98–103.
(6) Büsgen, M. (1891). *Jena Zeitschr. f. Naturwiss.* xxv, 340–428.
(7) Davidson, J. (1913). *Quart. Journ. Micros. Sci.* lviii, 653–701.
(8) —— (1914). *Journ. Linn. Soc. London (Zool.)* xxxii, 307–330.
(9) Dixon, H. H. and Atkins, W. R. G. (1914). *Sci. Proc. Roy. Dub. Soc.* 374.
(10) —— —— (1915). *Ibid.* 445.
(11) Grove, A. J. (1919). *Parasitology*, xi, 456–488.
(12) Guttenberg, H. von (1909). *Jahrb. f. wiss. Botan.* xlvi 453.
(13) Marchal, P. (1911). In Richet, *Dictionnaire de Physiologie*, 318 *et seq.*
(14) Morstatt, H. (1908). *Centrlbl. f. Bakt.* II, xxi, 408–424.
(15) Petri, L. (1908). *Centrlbl. f. Bakt.* II, xxi, 375–379.
(16) —— (1909). *Zeitschr. f. Pflanzenkr.* xix, 18–48.
(17) —— (1910). *Rendic. Accad. d. Lincei*, xix, 28–34.
(18) —— (1911). *Ibid.* xx, 57–65.
(19) Plateau, F. (1874). *Recherches sur les phénomènes de la digestion chez les Insectes.* Bruxelles, 1–124.
(20) Smith, K. M. (1920). *Ann. App. Biol.* vii, 40–55.
(21) Strassburger, E. (1921). *Text Book of Botany*, 5th English Ed. 1921.
(22) Williams, C. B. (1921). *Mem. Dept. Agric. Trinidad*, No. 1, 20–29.
(23) Woods, A. F. (1897). *Centrlbl. f. Bakt.* Abt. II, iii, 722–727.
(24) —— (1899). *Ibid.* v, 745–754.
(25) —— (1900). *U.S. Agric. Div. Veg. Physiol. Bull.* 19, 1–30.
(26) Zweigelt, F. (1914). *Centrlbl. f. Bakt.* Abt. II, xlii, 265–335.
(27) —— (1916). *Ibid.* xlvii, 408–535.

XI. EXPLANATION OF PLATES I AND II

PLATE I

Drawings showing the effects produced by *Aphis rumicis* on *Rumex* and *Vicia faba*. The staining methods employed are described in the text.

Fig. 1. Transverse section of *Rumex* stem, showing the piercing organ passing through the pericycle (*I*) into the phloem (*P*). Note the stylet sheath (*S*) (coloured red) surrounding the piercing organ. × 540.

Fig. 2. T.S. *Rumex* flower stem showing the piercing organ passing through the epidermis (*E*), cortex (*R*) and pericycle (*I*) into the phloem. Note the secretion of saliva (coloured red) at the tip of the piercing organ, and the displacement of the pericycle cells by that structure. × 500.

Fig. 3. T.S. of bean stem showing the course of the stylet track (coloured red) and the effect of the saliva on the cells. Note the contents of the stylet sheath forced out on the surface of the epidermis. × 500.

Fig. 4. T.S. of *Rumex* flower stem showing the stylet tracks in the pericycle (*I*) and phloem and the penetration into the xylem vessels. × 540.

PLATE II

Drawings showing the effects produced by ***Aphis rumicis*** on *Vicia faba*, and ***Rumex***; ***Myzus cerasi*** on the peach and ***Macrosiphum rosarum*** on the rose.

Fig. 1. T.S. of leaf petiole of peach showing destruction of the cells and the secretion of saliva in the phloem region. x 500.

Fig. 2. T.S. of young bean stem showing intercellular course of the stylet track. Note the irregular globules of saliva in the intercellular spaces. x 500.

Fig. 3. T.S. through bean stem showing intracellular stylet track in the phloem, leading into a companion cell. x 540.

Fig. 4. Radial section through a rose stem showing the piercing organ of ***Macrosiphum rosarum*** passing through the tissues to the phloem and entering a sieve tube. Note the stylet sheath (coloured red) surrounding the stylets.

Fig. 5. T.S. of ***Rumex*** stem showing stylet track passing along the middle lamella of the cell walls in the pericycle. x 540.

REFERENCE LETTERING.

S = stylet sheath. *P* = phloem. *I* = pericycle. *R* = cortex. *E* = epidermis. *M* = xylem. *O* = piercing organ. *G* = starch grains. *T* = pith cells. *X* = maxillary stylets. *D* = mandibles.

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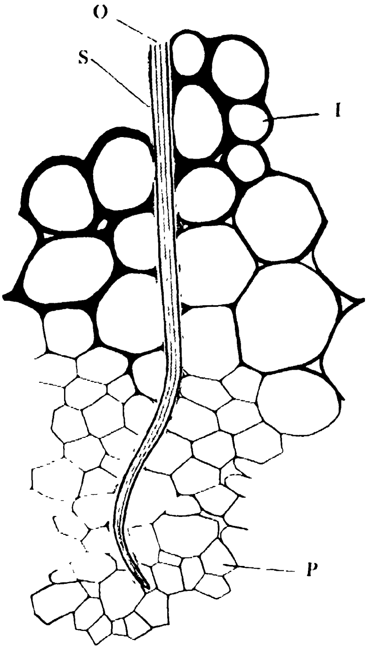

Fig. 1

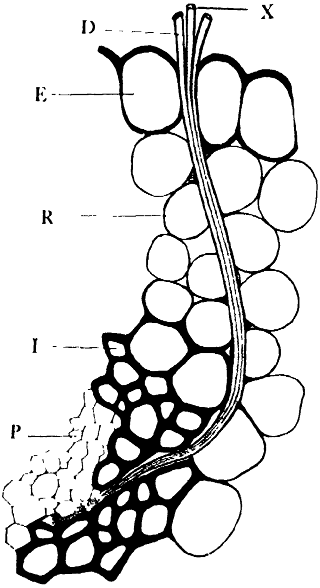

Fig. 2

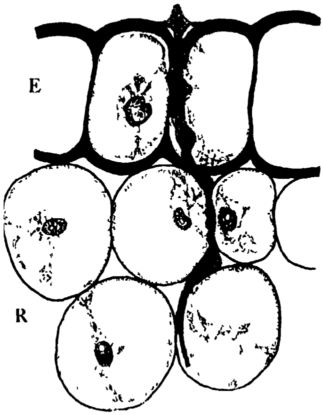

Fig. 3

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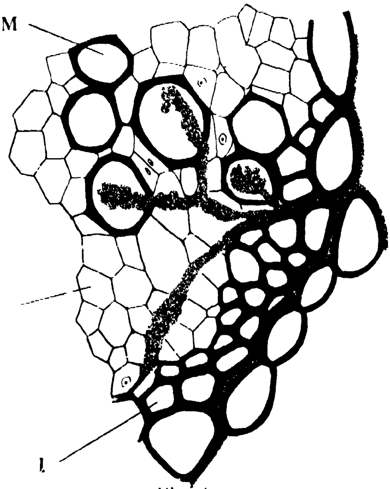

Fig. 4

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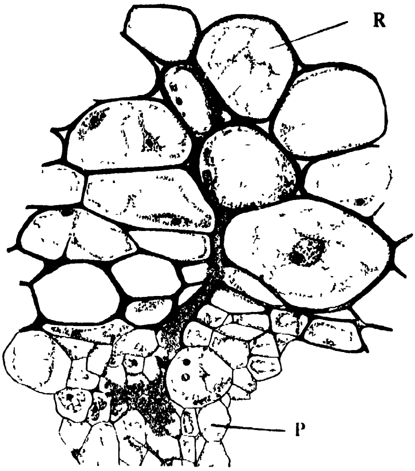

Fig. 1

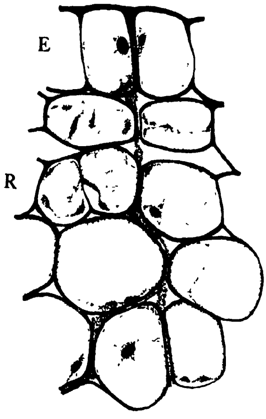

Fig. 2

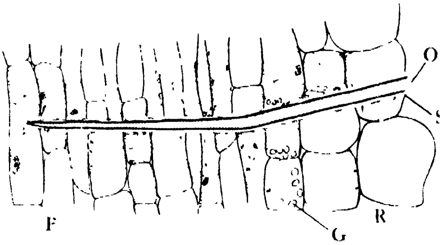

Fig. 4

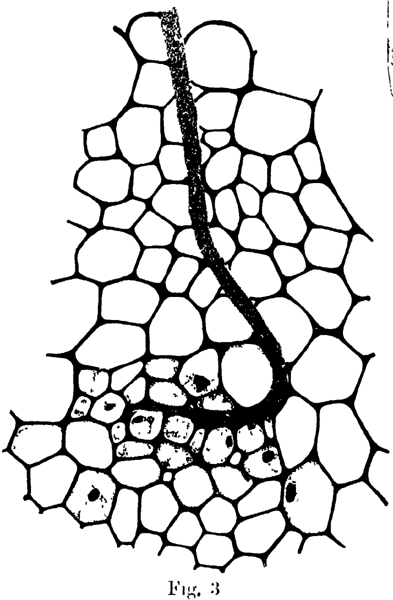

Fig. 3

J. D. del.

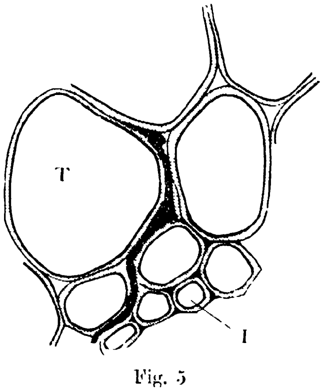

Fig. 5

"VIRUS DISEASES" OF ANIMALS

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THE first disease attributed to a "filter-passing virus" was Mosaic disease of the tobacco plant which was described by Iwanowski in 1892 (11). The next, namely, Foot and Mouth disease of cattle was investigated by Loeffler and Frosch (14) and their observations on the virus were recorded in 1898, the same year in which Beijerinck (4) published his rediscovery of the virus of Mosaic disease. Since then, however, the animal viruses must have far outrun those affecting plants in number, since about 48 or 50 are already on record.

The chief points of interest common to virus disease of animals and plants appear to me to be

(1) The nature and properties of the virus.
(2) The means of transmission.
(3) The means which can be adopted for prevention.

The usual synonyms for a "virus disease" have been a disease caused by a filter-passing organism or by an ultra-microscopic virus. In what follows, however, the term "virus disease" is taken to mean an infective disease which can be transmitted experimentally, though it is not due to any known protozoon, spirochaete or ordinary bacterium, but can be transmitted by a liquid containing only very minute particles—smaller than ordinary well-known bacteria. This definition is not very exact, but the boundaries of the group are so ill-defined that it is impossible to cut it off by a sharp line.

The group includes a large number of "viruses" most of which may be classified as filter-passers, but also admits some which will not pass through a Chamberland or even a Berkefeld filter when suitable precautions are taken to prevent the smallest cultivable bacteria such as *B. pyocyaneus* or *B. prodigiosus* from passing. Filtration through a so-called "bacterial" filter is not a very satisfactory criterion of size, since much depends on the rate and duration of the filtration and on the pressure used, as well as on the readiness with which the virus is adsorbed on to the surfaces of the filter. The consistency of the liquid in which the virus is contained is also of great importance, for with a thick viscous colloidal suspension or undiluted blood serum, the pores of the filter rapidly become narrowed and blocked. Moreover, the smallest members of a young culture of some bacteria are probably as small as the larger individuals of some of the coarser viruses, *e.g.* *Rickettsia*, the presumptive causal organism of Typhus and Trench fevers, and the minute bodies seen in cultures and described as the micro-organisms of Influenza and Fowl diphtheria, which are believed by their sponsors to be filterable.

To examine critically the filterability of a virus it must be compared with a known emulsion of small bacteria. Both should be tested with the same filter under as nearly as possible the same conditions of time, pressure and temperature and in a liquid as nearly as possible of the same composition or consistency.

If, on the other hand, the visibility of the active agents in a virus is to be taken as a criterion for classification, the difficulties are not much less. Almost all the diseases which I am considering are caused by viruses whose microscopic appearances are either quite unknown or in dispute; in a number of instances the morphology of the organism has been described but either its pathogenicity has not been tested by animal inoculation, or culture and inoculation, or else claims to have done this with success are not generally accepted.

Since both the above criteria—filterability and visibility—depend to a large extent on the size of the particles composing the virus it is necessary to consider what order of size these methods are concerned with. *The size of particles*, which will pass a Chamberland filter (*F*) or a good Berkefeld is generally stated at about 0·2 micron in diameter (0·0002 mm.) (Rosenthal). This approximates very closely to the size of particles which can be clearly resolved with the high power of the microscope, which is put at 0·15–0·2 micron. It has been calculated (8) that the smallest living organism cannot be much less than 2 micro-microns (2/1000 micron) and would probably be not less than 5/1000 micron in diameter. This calculation is based on the size of molecules large enough to contain the constituents of living protoplasm (proteins and salts). That is to say, a living organism can be conceived, of which the diameter is 1/40 of that of the smallest clearly visible particle. This is a disputable calculation except as a minimum. The ultra-microscope is capable of revealing particles only 4–5 micro-micron (4/1000 to 5/1000 micron) *i.e.* 1/50th of the smallest clearly visible particle and about the same size as the smallest conceivable living organism. The ultra-microscope, however, gives no certain information with regard to size or shape and, therefore, does not enable minute micro-organisms to be distinguished from unorganised particles. A further property viz. diffusibility has been attributed to some viruses, *e.g.* of Rabies in glycerine (17) and of Mosaic disease in agar jelly (4). Since the former of these viruses is not very readily filterable through a Berkefeld, it seems unlikely that in its usual form the virus is truly diffusible, but this question awaits further work.

It is seen then, that it is not at all an easy matter to define the properties of the pathogenic viruses, and classification of them is still more difficult and really a matter of rough lists which may be used for comparing them in studying their properties. A good classification is not possible at present as so few of their properties are clearly ascertainable and viruses which agree in one particular, *e.g.* resistance to glycerine, like Vaccinia, Poliomyelitis and Rabies, differ in other respects, *e.g.* readiness with which they pass through a bacterial filter. And those which pass through a porcelain filter, *e.g.* the viruses of Foot and Mouth disease, Horse sickness, Poliomyelitis and Encephalitis lethargica differ in regard to their resistance to drying, exposure to phenol, glycerine etc.

It must be remembered that the diseases in this group are thrown together partly because so little is known about their causation and from time to time so-called filter passers are found to possess distinctive and clearly recognisable pathogenic microbes which are not at any rate in all conditions so extremely minute. For instance, the serum of patients containing the virus of Yellow fever was stated to pass a Chamberland filter *F* in an undiluted state and a finer Chamberland *B* when diluted by Rosenau, Parker etc., whereas, more recently Noguchi and others appear to have shown that true Yellow fever is due to a slender spirochaete (*Leptospira icteroides*) of about 0·2 micron diameter but very much longer.

The diseases under consideration may be roughly divided into four classes:

Class I. The largest; visible, not filterable, not cultivated.
,, II. Intermediate; probably visible, filterable, cultivated.
,, III. The least in size and the most inscrutable; filterable, not cultivated (*a*) visible in animal tissues, (*b*) not visible.
,, IV. Filterable, very resistant.

It must be understood that these attributes are assigned to the different viruses on a balance of evidence, and that such a term as "visible" only means that there is good reason to believe they can be or have been clearly seen under a high power.

Table I.

Classification.

I	II	III		IV
Visible	Visible	Filterable		Resistant
Not filterable	Filterable	Not cultivated		? Visible
Not cultivated	Cultivated	Visible?	Not visible	Not cultivated
(Rickettsia)		(strongyloplasmata)		
Typhus Trench fever Rocky mountain spotted fever	Pleuro-pneumonia of cattle Avian diphtheria Fowl-plague? Influenza Poliomyelitis	Rabies Sheep-pox Fowl-pox Fowl-plague? Vaccinia Variola Molluscum contagiosum Trachoma Scarlatina	Foot and mouth Rinderpest Swine fever Distemper Dengue Fowl-leukaemia Mumps Measles	Infectious anaemia of horses Fowl-pox Fowl-sarcoma Horse-sickness

Class I. The virus is probably not filterable through a Berkefeld which will hold back ordinary small bacteria, but it has been considered filterable at some time by a competent observer. Forms of about 0·2–0·3 micron in diameter which are believed to be the pathogenic micro-organisms can be seen and identified in stained films. This group includes Rocky Mountain spotted fever, Typhus fever, and Trench fever. Claims have been made that the forms known as *Rickettsia* and seen in the virus of Typhus and Trench fever have been cultivated on artificial media from patients and lice infected with Typhus but this is not yet generally accepted.

Class II. Viruses which are intermediate in their known properties, and are related to certain diseases, *e.g.* Pleuro-pneumonia of cattle, Poliomyelitis or Infantile paralysis, Influenza and Avian diphtheria. The virus of each of these it is claimed has been filtered and cultivated, the pathogenic organisms seen in stained films of the cultures and the diseases reproduced with subcultures far removed from the original material. The minute organisms described in Poliomyelitis, Avian diphtheria and Influenza appear to closely resemble the strongyloplasmata of Lipschütz(13) which are included in Class III.

Class III. Comprises a large number of diseases which are transmissible by a virus which is believed to pass a Berkefeld or Chamberland, but with regard to some of them there is difference of opinion or rather in experience of different workers, and there is no doubt that the ease with which an active filtrate can be obtained is very variable among the members of the Class. In section (*a*) of Class III very small bodies named strongyloplasmata by Lipschütz(13) have been seen in the tissues, inside cells and also in the virulent discharges and have been credited by Prowazek(16) and Lipschütz(13) with being the causes of the diseases. These minute bodies are about 0·15–0·2 micron in diameter, are rounded or dumbbell-shaped as a rule and occur in very large numbers. In speaking of strongyloplasmata Lipschütz says(13) "In the affections under consideration (Vaccinia, Variola, Sheep-pox, Molluscum contagiosum, Trachoma, Fowl-pox, etc.) the morphology of the causal organisms is so similar and uniform that it must be looked upon as a remarkable freak of nature that micro-organisms which can scarcely be distinguished should have such extraordinarily diverse biological properties" (Kolle and Wassermann, 2nd Ed. VIII, p. 365). Prowazek called these minute forms elementary bodies and considered them a stage in the life history of the pathological micro-organism which was represented at other stages by larger bodies which he called initial bodies and much larger latent forms which he saw in the inclusion bodies in cells such as the Negri bodies of Rabies, and the Trachoma inclusions. The elementary bodies are described as usually intracellular, but may be intranuclear, *e.g.* in *Virus myxomatosus* and *Gelbsucht*, or extracellular (Trachoma) or in cell-free fluid as in Pleuro-pneumonia of cattle.

The following 10 or 11 diseases which it is claimed are associated with the Chlamydozoa or Strongyloplasmata and in which the pathological changes are attributed to these bodies are

Fowl-plague (Prowazek).
Fowl-epithelioma or Fowl-pox (Borrel).
Sheep-pox (Borrel and Paschen).
Vaccinia (Prowazek and Paschen).
Molluscum contagiosum (Lipschütz).
Variola (Prowazek).
Trachoma (Halberstadter and Prowazek).
Scarlatina (Paschen, Bernhardt and Cantacuzene).
Virus myxomatosus of rabbits (Beaurepaire-Arago).
Alastrim.
Gelbsucht (Prowazek).

The appearance of these bodies (strongyloplasmata) very much resembles some forms found in cultures of poliomyelitis virus (Flexner and Noguchi), common Cold (Foster), Influenza (15), Fowl diphtheria (6) etc. so that the claim to have cultivated these latter has encouraged work with a view to the cultivation by similar methods of the strongyloplasmata. There has been and still is a great deal of hesitation about accepting the strongyloplasmata as pathogenic organisms and this is extended in a less degree towards the very minute somewhat similar forms seen in cultures and for the same reasons.

This is due largely to the difficulty of recognising such very small objects which are often stained with difficulty and of distinguishing those associated with a given disease from similar bodies found in other diseases and also from mere precipitates and globules in emulsions of colloidal substances which occur in smears of animal tissues and in certain culture media especially those containing serum or blood after a few days' incubation. Most of these stain red with Giemsa's stain and resemble strongyloplasmata very closely. It has recently been suggested by Woodcock (20) that these are all the result of red blood cell or nuclear disintegration.

The recognition, identification and differentiation of these very small forms under a high power of the microscope is then very difficult and at first appears impossible. Experience, however, in searching for the very slightly larger and more definite Rickettsia-bodies of Typhus and Trench fevers has taught one that possibly the difficulty may not be insuperable with practice, if the range of size and shape and the arrangement of the elements are specially considered, and if a large number of the forms are viewed, so that too much importance is not attached to the individual forms. This is particularly important since in all or almost all these very small bodies in viruses and even in the case of *Rickettsia* the prevailing shapes are round, dumbbell or diplococcal, and similar shapes are taken by precipitated or finely divided protein or other colloidal matter.

When, however, a large collection of these forms is examined it may be seen that as in the cases of *Rickettsia quintana*, the round and very short forms are almost invariably present in greatly preponderating numbers, whereas *R. Prowazeki* takes on longer and even thread-like forms and shows a much greater range of variation in size, especially under certain conditions, and it is usually intracellular. Moreover, *R. quintana* stains a purplish colour with Giemsa and *R. Prowazeki* stains a much redder colour. In the same way more variation in form and much larger individuals are seen in films of *R. melophagi* though the commonest forms are rounded and stain purple like *R. quintana*. *R. lectularii* is intracellular in some stages and also forms long threads; it stains red with Giemsa.

While it is perhaps necessary to preserve a rather sceptical attitude towards the strongyloplasmata as causes of disease, still their possible importance cannot be hastily set aside especially as evidence is forthcoming that very similar forms can be cultivated in artificial media and reproduce the disease from which the material started.

The position seems to be that in some cases films showing strongyloplasmata or culture forms resembling them are described and demonstrated by one observer whereas others are unable to distinguish the bodies in question from similar objects found in uninoculated culture media, blood, etc.

I confess that so far this latter has been my position with regard to preparations from common Cold, Influenza, Vaccinia, Foot and Mouth, and some others which I have had the opportunity of seeing.

For instance, in attempts by the writer to culture the virus of Trench fever very large numbers of round and dumbbell-shaped minute bodies were found in films of the culture tubes after incubation. These usually stained red with Giemsa. They were in some instances extremely numerous and had a certain resemblance to *Rickettsia* and at the time were not seen in preparations from control culture tubes of culture media. The appearances were so striking that it was thought justifiable to try and reproduce the disease by inoculation, but the attempt failed and later many forms were found in the control tubes which we were unable to distinguish from those in the supposed cultures.

In the same way "lymph" from a "foot and mouth" vesicle on several occasions appeared full of minute strongyloplasmata. The suspicion was heightened by the belief that the lymph contained a very concentrated virus. These forms were not, however, always found in virulent lymph and they, moreover, resembled granules seen in uninfected tissue material and in attempted but inactive cultures. The picture seen in the virulent lumph very closely resembled the plates given by V. Betegh (5) of forms believed by him to constitute the virus (see also Huntemuller's observations on F. and M. lymph).

The conditions necessary for a presumption of causal relationship of a given body or bodies, seen under the microscope but not cultivated, to a disease must include quite definite evidence that the disease and the microscopic bodies are associated in a high proportion of cases examined and that the forms seen are not found under similar conditions apart from the disease. That the bodies are so characteristic as not to be liable to confusion with other forms seen in other diseases or under similar circumstances is a necessary part of the evidence.

If these criteria are fulfilled a close association of the microscopic bodies and the disease is proved and a presumption of causal relation is established.

In practice, the degree of probability for this connection depends very much on the certainty with which the disease in experimental animals and the microscopic forms can be identified, the number of cases and controls examined and the number of cases in which association occurs. In many of the investigations into virus diseases the observations have been very few. The best evidence of the causal relationship of a microscopic form to a disease is obtained by culturing the forms on artificial media through a number of generations and then reproducing the disease in an animal in a clearly recognisable form. Evidence of this kind has been produced in the case of Pleuro-pneumonia of cattle and Poliomyelitis.

In the case of the Influenza virus which under the name of *Bacterium pneumosintes* has been recently cultivated by Olitsky and Gates (15) and in a somewhat similar way by Gordon (9) the disease produced in animals is not so like the original disease in man as to be certainly the same. This is very likely in part due to the want of a suitable experimental animal. One of the most serious obstacles to proving the existence of activity of a virus in culture is the high degree of natural infectiousness of the virus diseases, such as Influenza, Foot and Mouth, Distemper, etc., rendering the isolation of control animals extremely difficult.

Another line of criticism which has to be met is that although the virus may be present and increase in cultures still the *visible forms may not be the true virus* but are formed by the virus or are only incidentally associated with it. Even when the case is a much easier one, *i.e.* when the visible forms are undoubted bacteria, it may be only possible to separate them from the virus by filtration and they may be constantly associated with the virus in diseased animals, although the filterable virus is the primary pathogenic agent and the bacterium is only a secondary invader. This is the case in Swine fever in which the *B. suipestifer* is very frequently present and produces a secondary infection and was therefore at one time accepted as the only cause of the disease on account of its supposed constant presence in disease and its undoubted pathogenicity. The filterable virus of Poliomyelitis (Flexner and Noguchi (7)) and the associated streptococcus are another puzzling instance of associated virus and bacterial infection. Also in Influenza the pneumococci and streptococci very frequently caused secondary infections and it is disputed whether the disease is due to the *B. influenza* or to a filter-passer with the *B. influenza* as a superadded infection. If an ultra-microscopic filterable virus is constantly associated with forms resembling stronglyoplasmata the bacteriologist who attempts to prove the identity or diverse nature of the visible forms and virus needs a stout heart and a long life.

GENERAL CHARACTERS OF VIRUSES.

It seemed at first when filter-passing viruses were discovered that they must have some intangible, indefinable consistency necessitating a new and strange conception of contagion. This is expressed in Beijerinck's phrase *Contagium vivum fluidum*. There has been a tendency to credit the filter-passing viruses as a whole with greater resistance to chemical and physical agents than most bacteria. The tests have as a rule been made with virus contained in the blood, secretions, excretions or tissues, in which it is found in disease. This fact must be taken into account if comparison is made with cultures of bacteria in broth or emulsions of cultures in salt solution, and, moreover, the very high concentration in which some of these viruses must occur has to be reckoned with. It has been stated for instance on high authority, that one millionth of a c.c. of blood etc. from a bird with Fowl-plague or one hundred-thousandth c.c. of the contents of a Foot-and-mouth disease vesicle will transmit the disease.

Few comparative tests of resistance and viability appear to have been made on filter-passing viruses and bacteria as far as possible under similar conditions.

Table II.

Resistance of Virus.

Disease	Glycerine	Drying	Heat	Carbolic
Vaccinia	203 days at 10° C +	220 days +	60° C. 20 mins. –	...
Rabies	10 months +	4–15 days –	50° C. 1 hr. –	1 % 2–3 hrs.
Fowl-plague	270 days +	20 days at 22° C. +	Liver 62° C. 30 mins. – Brain 65°–68° C. 4 hrs. +	...
Pleuro-pneumonia	+	...	58° C. –	...
Poliomyelitis	5 months +	20 days +	56° C. –	1 % –
Fowl-pox	170 days +	1½ years +	60° C. dry 3 hrs. +	1 % –
Horse-sickness	...	–	45° C. +	3 % +
Swine fever	...	3 days +	60° C. –	...
Rinderpest	8 days –	...	58° C. –	1 % –
"Foot and mouth"	–	...	50° C. –	1 % –
Sheep-pox	12 days –	...	...	...
Typhus	–	Blood –	55° C. –	...
Trench fever	–	Blood – Louse faeces +	55° C. –	...
Infectious anaemia of horses	...	7 months +	58 C. –	...

+ = persistence of virulence. – = loss of virulence.

Though the characteristics as regards resistance are by no means uniform among filter-passers causing animal diseases, it does not appear that they are very different from those obtaining among bacteria. Thus most viruses are destroyed at 55 to 60° C. Drying is destructive of some and not of others; glycerin, chloroform, ether and carbolic as well as solutions of bile and saponin are rapidly fatal to some viruses but not to all. It must be conceded that certain viruses, *e.g.* Vaccinia, appear to be far more resistant to glycerin, chloroform and ether than are most non-sporing bacteria. The three last mentioned agents do cause a deterioration or reduction in the virulence or perhaps numbers of the individual organisms in the virus, but the reduction in the number of the non-sporing bacteria seems to be much more rapid, perhaps partly because their number is vastly smaller.

The different effect of drying according to the concentration of the virus (the number of its elements) is probably illustrated by the different effect of drying the virus of Typhus or Trench fever in the blood of a patient and in the excreta of lice. The excreta are far more virulent and contain enormous numbers of Rickettsia-bodies so that less than 1 mg. will cause the disease in a guinea-pig, whereas 2 c.c. of blood is usually required. The louse excreta resist moderate drying whereas blood when once dry appears to have lost its efficacy. There is no other reason to suppose that a different form of virus is present in blood and lice. Associated with the view that the filterable viruses were of a different nature from bacteria or protozoa on account of their resistance the idea was entertained that many of them were very much smaller than the smallest bacterium and were perhaps only visible with the ultra-microscope. The tendency at the present day is to think that in many cases the virus is identical with very minute visible particles. This is encouraged by recent apparent successes in culture, and at any rate appears to be the fashionable view.

No virus of an animal disease appears to be so resistant to heat in a moist condition as are the juices of plants infected with Mosaic disease (70–80° C.) nor will they survive treatment with alcohol as is recorded of the Mosaic virus.

The virus of Bird-pox or Avian epithelioma is amongst the most resistant to drying, heat (60° C. for a short period), and glycerin; the virus of Fowl-plague in brain tissue is stated by Prowazek to resist 65 to 68° C. for four hours as well as desiccation over KOH at 22° C. for 20 days. The viruses of the Infectious Anaemia of horses, Fowl-plague, and Horse sickness are said to remain active amongst decomposing dung for many days. Rabies virus has been found to be still active after exposure to 5 per cent. carbolic for 24 hours. The filterable virus of Fowl-sarcoma in its resistance to drying for long periods resembles these.

The remarkable properties of some of these unusually resistant viruses have suggested that an *unorganised ferment or enzyme* might in some cases act as a virus. The question has been debated since Iwanowski discovered in 1892(11) and Beijerinck(4) rediscovered and discussed the nature of the filterable virus of Mosaic disease. The suggestion that the virus of an infective disease might be an enzyme has usually been rejected as *a priori* untenable since an enzyme cannot be supposed to reproduce itself. Beijerinck called the virus of Mosaic disease a *contagium virum fluidum*, but appreciating the difficulties of this suggestion he apparently had an alternative or accessory view that the virus was not strictly speaking living on its own account for he proposed that it might enter the cells of the infected plant and become in such a way united with the contents of the cells that when the latter divided the virus might be taken along and divide also with them. This I think implies that something like an enzyme is so tacked on to the living substance of the cell that the cell protoplasm becomes modified and then grows and divides while retaining its new additions which then wholly or in part can be shed or excreted as an independent enzyme.

This is a bold hypothesis but something like it has recently been advocated in a rather different form by Bordet to explain the reproduction or multiplication of d'Herelle's so-called Bacteriophage. It seems possible that the phenomenon of Twort and d'Herelle may throw light on the nature of some of the filterable viruses of infective disease of animals, and it may be best to briefly recall the facts and the explanations which have been put forward.

The phenomenon, as is well known, consists, at any rate as far as concerns our present purpose, in the fact that has often been verified by many observers that when a Berkefeld filtrate containing the active principle has once been obtained it can be multiplied indefinitely by adding a few drops of the filtrate to a tube or flask full of a young culture of susceptible bacteria, *e.g. B. dysenteriae* (Shiga). These latter are dissolved and every drop of the contents of the tube or flask after filtration is found to have become as active, *i.e.* to contain as potent and concentrated a solution as the original drops introduced. This implies indefinite multiplication of the lytic agent, but does not necessarily mean that the "bacteriophage" reproduces itself as a living organism. The active liquid besides passing through a bacterial filter has been found to resist a temperature of 75° C. without destruction.

It is d'Herelle's opinion that the "bacteriophage" is a living microbe and the facts observed by him and others certainly appear to afford considerable analogy with the filterable viruses of some diseases of higher animals and plants. Almost all other workers beside d'Herelle dispute his conclusions for two reasons.

(1) The liquid containing the lytic agent resists high temperatures, 75° C., and also chloroform and other antibacterial substances.

(2) The phenomena, though recently presented in a new form by Twort and d'Herelle resemble very closely those which have long been known under the name of *autolysis* and it seems probable that the Bacteriophage will be found to be explained by the action of autolytic ferments, *e.g.* such as that derived from the meningococcus and described by Flexner in 1907.

The two hypotheses proposed to account for the phenomenon without postulating a living virus may be briefly stated as follows.

I. Bordet suggests that the phenomena are due to a soluble substance which diffuses into the bacteria and then participates in their division and multiplication and becomes in fact an hereditary factor; the hypothetical substance being thus both *contagious* and *hereditary*. This appears to be very like the hypothesis of Beijerinck.

II. The alternative and more generally accepted view is that the so-called "bacteriophage" is really of the nature of a lytic enzyme which so affects the bacteria in a culture that they break up and in so doing liberate large quantities of autolytic ferment which in turn act like the original lytic substance. This view though not without its difficulties appeals strongly to the present writer.

If an autolytic enzyme originating from a bacterium and able to destroy living organisms of the same species is admitted as the explanation of the d'Herelle phenomenon, then it seems no longer possible to deny *a priori* that an unorganised ferment acting in a similar way on the cells of the animal body might destroy them and in so doing induce the liberation of a fresh supply of a similar ferment. The ferment would then be multiplied or reproduced by the cells of the body though strictly speaking it could not reproduce itself in the sense that a living organism does. The difficulty in conceiving how an infectious disease could be due to a ferment would then resolve itself into the difficulty in understanding how the hypothetical enzyme could be conveyed from one host to another. This does not seem an insuperable obstacle since the ferment might be transmitted in the same way as living organisms by dust, droplets, etc. Such an hypothesis as the existence of an enzyme-virus to explain certain filter-passing infectious diseases is perhaps not necessary and much further work on the more resistant viruses would be needed before the existence of a living pathogenic microbe could be definitely excluded.

A very short reference must be made to other points of resemblance between the diseases of animals and plants of the kind now under discussion. As such instances of resemblance may be mentioned:

(1) Carriers of disease in the sense of recovered or unrecognised cases of disease which are infective and act as sources and stores of virus from which infection spreads. Such carriers are recognised in Foot and Mouth disease, Trench fever, Influenza, Poliomyelitis, etc.

(2) Insect vectors, *e.g.* biting flies (horse-sickness), mosquitos (Dengue fever), lice (Typhus and Trench fevers), ticks (R.M. Spotted fever). The larva of the kedani mite or harvest-bug (Trombicula) conveys Flood fever or Tsutsugamushi. In most of these diseases, if not in all, a period of incubation in the insect is necessary. In the same way I see that Mosaic disease is believed to be carried by aphides.

(3) Another point of resemblance is that special cells are said to be attacked and appear to constitute the sites of multiplication of the virus.

It has been often observed that many of the animal viruses appear to have a special affinity for certain organs or cells of the body. Lipschütz divides the virus diseases into two main groups.

Group A. Acute general infections comprising 18 diseases.

,, B. Containing 21 localised diseases, of which 3 attack the epidermis, *e.g.* Molluscum contagiosum, Trachoma; 6 the skin, dermotropic, *e.g.* Variola, Foot-and-Mouth; 6 neurotropic, *e.g.* Rabies and Poliomyelitis; 2 haemotropic, *e.g.* Fowl-leukaemia; 4 organotropic, *e.g.* Pleuro-pneumonia and Mumps.

In several of the latter groups Prowazek, Lipschütz and others have described strongyloplasmata or larger bodies in the special cells. *Rickettsia prowazeki* of typhus lives and multiplies in the intestinal cells of the body louse and according to Wolbach and Todd also in the endothelial cells of the small vessels of mammals infected with typhus.

It is, therefore, of interest that Iwanowski (1902) recorded the occurrence of very minute bacteria filling certain cells in Mosaic disease; I do not know, however, to what extent this has been confirmed.

(4) Prevention depends on the same principles in plants and animals except as regards means taken to produce immunity in individuals.

Prevention consists in (i) finding and selecting strains resistant to disease, which has occasionally proved of value in animals but is probably far more important in plants; (ii) isolation or destruction of cases of disease; (iii) raising the resistance of individuals by feeding and improved hygiene, fresh air etc.; (iv) isolation or destruction of carriers of the virus, when the carriers are not so numerous as to render this impracticable; between and at the commencement of epidemics this may be effective; (v) destruction or avoidance of insects or other vectors.

CONCLUSIONS.

1. The so-called "viruses," with very few exceptions are not very different in their properties from a bacterial *materies morbi.*

2. Apparent differences are partly accounted for by the extreme concentration of some samples of virus and the difficulty of comparing the resistance of virus and bacteria under comparable conditions.

3. There is very strong evidence that very small micro-organisms are present in very large numbers in various cells, organs and secretions during some of the so-called virus diseases and that they can be cultivated, obtained in pure culture and, after several passages, reproduce the disease.

4. Some viruses pass through a fine Chamberland filter, but with a considerable loss of strength or virulence. It does not follow that the forms which are seen are identical and of the same size with those which pass through a filter.

5. The difficulty of establishing the causal connection between microscopic bodies of 0·2–0·3 micron and a disease is largely due to difficulties of culture, but the recognition by staining properties and morphology of such minute bodies and the differentiation of those belonging to different diseases from each other and from precipitates and other non-living colloidal particles is also at present an extremely hazardous performance.

6. The analogy with diseases such as Rocky Mountain fever, Typhus and Trench fevers which are associated with definite bodies (Rickettsia) which can be differentiated morphologically suggests that similar differentiation in the case of some particles associated with other diseases, may in time be achieved. The evidence regarding Influenza and some other virus diseases has been strengthened lately but can hardly yet be regarded as conclusive.

7. That an unorganised ferment may cause infective disease does not seem to be theoretically impossible on *a priori* grounds, especially if the phenomena of the lytic substance of Twort and the bacteriophage of d'Herelle are considered as analogous cases.

8. The better known and most thoroughly investigated virus diseases of animals do not seem to need any hypothesis other than that of a

minute micro-organism not unlike a very minute *bacterium* to account for their characteristics and those of their viruses as far as these are known.

9. A few viruses, *e.g.* those of Fowl sarcoma, Horse pernicious anaemia, and Fowl-pox have characters attributed to them which seem to belong rather to an enzyme than a living micro-organism. The alleged diffusibility of Mosaic disease and rabies viruses also suggests a soluble virus.

REFERENCES.

(1) ARKWRIGHT, J. A., BACOT, A. and DUNCAN, F. M. (1919). The minute bodies found in association with Trench fever, Typhus fever and Rocky Mountain spotted fever. *Trans. Roy. Soc. Trop. Med. and Hyg.* XII, 61.

(2) ARKWRIGHT, J. A., ATKIN, E. E. and BACOT, A. (1921). An hereditary Rickettsia-like parasite of the Bed-bug (*Cimex lectularius*). *Parasitology,* XIII, 27.

(3) ARKWRIGHT, J. A. and BACOT, A. (1921). Stages of Rickettsia in the Sheep-ked, *Melophagus ovinus. Trans. R. Soc. Trop. Med. and Hyg.* XV, 146.

(4) BEIJERINCK, M. W. (1899). Ueber ein Contagium vivum fluidum als Ursache der Fleckenkrankheit der Tabaksblätter. *Centralbl. f. Bakt.* Abt. II, v, 27.

(5) v. BETEGH, L. (1911). Beiträge zur Aetiologie der Maul- und Klauenseuche. *Centralbl. f. Bakt.* Abt. I, LX, 86.

(6) BORDET, J. and CIUCA, M. (X, 1920). La bactériophage d'Herelle, sa production et sa interprétation. *Compt. r. de Soc. de Biologie,* LXXXIII, 1296.

(7) FLEXNER, S. and NOGUCHI, H. (1913). Experiments on the cultivation of the micro-organism causing Epidemic Poliomyelitis. *Journ. Exp. Med.* XVIII, 461.

(8) FREI, W. (1920). Zur physikalische Chemie der filtrierbaren Krankheitserreger. *Arch. f. wissenschaftl. u. praktische Tierheilkunden,* XLVI, 103.

(9) GORDON, M. H. (1922). The filter-passer of Influenza. *Journ. of R.A.M.C.* XXXIX, July, p. 1.

(10) D'HERELLE (1919). Sur le microbe bacteriophage. *Compt. r. de Soc. de Biologie,* LXXI, 1237.

(11) IWANOWSKI, D. (1892). Ueber die Mosaikkrankheit der Tabakspflanze. *Centralbl. f. Bakt.* Abt. II, v, 250.

(12) —— (1902). Ueber die Mosaikkrankheit der Tabakspflanze. *Centralbl. f. Bakt.* Abt. II, VII, 148.

(13) LIPSCHÜTZ, B. (1913). *Handb. der pathogenen Mikroorganismen.* Jena, 2nd ed. VIII, 345.

(14) LOEFFLER and FROSCH (1898). Berichte der Kommission zur Erforschung der Maul- und Klauenseuche bei dem Institut für Infektionskrankheit in Berlin. *Centralbl. f. Bakt.* Abt. I, XXIV, 371.

(15) OLITSKY and GATES (1922). *Experimental studies of the Nasopharyngeal secretions from Influenza patients. Journ. Exper. Med.* XXXV, 813.

(16) PROWAZEK, S. *Handb. der pathogenen Protozoen.* Leipzig, I, 1912; II, 1920.

(17) REMLINGER (1919). La diffusibilité du virus rabique. *Ann. de l'Institut Pasteur,* XXXIII, 28.

(18) TWORT, F. W. (1915). An investigation of the nature of ultramicroscopic viruses. *Lancet,* 4 Dec., 1241.

(19) —— (1920). Researches on Dysentery. *Journ. Exp. Pathology,* I, 237.

(20) WOODCOCK, H. M. (1921). An introduction to the study of haematophagy. *Journ. of R.A.M.C.* Nov. and Dec.

N.B. The greater part of the bibliography of the subject will be found in the article by Lipschütz and the handbook of v. Prowazek which are referred to above.

COMMON SCAB OF POTATOES[1]

PART II.

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(With Plates III and IV.)

INTRODUCTION.

An account of some experiments on Remedial Measures for Common Scab has been given by the writer in a recent Report (8). It was there shown that the disease may be inhibited by green-manuring and that, where it is possible to apply a sufficient quantity of green organic matter and to work this into a very intimate mixture with the soil, scab may be entirely prevented. One photograph illustrating this result (Pl. III, Fig. 1) is reproduced from the report. In the experiments described, the green manuring was carried out, for the most part, by the addition of grass cuttings, but this treatment, though simple to apply in the case of a garden or allotment, is obviously impracticable for potato cultivation on a farm. Here, the process must necessarily consist of ploughing in a green crop. The first section of this paper describes some trials of the green-manuring treatment carried out in this way, and gives an account of some further experiments in which hay and spent hops were tried as substitutes for green crops.

The second section deals with the action of liming on scab and the counteraction of its effect by green-manuring. Much attention has been given of late years to the relation of the soil reaction with the incidence of scab and the action of lime on scab must obviously yield valuable evidence in any discussion of this subject. In the third section, an attempt is made to show that the deductions previously drawn from such evidence are fallacious and the various theories put forward to account for the occurrence of scab and its cure by green-manuring are considered.

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[1] A grant in aid of publication has been made for this communication.

SECTION I. FURTHER EXPERIMENTS ON GREEN-MANURING AND ON THE USE OF SUBSTITUTES FOR GREEN CROPS.

These were carried out with the object of ascertaining whether the practice of green-manuring, as it obtains in ordinary farm practice, would suffice of itself to inhibit scab, and also whether such substitutes as old hay and spent hops would exert a similar effect.

Green-manuring.

Exp. 1. A plot 22 ft. × 6 ft. was divided into two parts. On one half, three successive crops of mustard were grown and dug in during the summer of 1919 and these were followed by a crop of rye which was dug in in the following spring. Potatoes were then planted on the whole plot, artificial manures only being applied.

The amount of scab on the fallowed half of the plot was unfortunately rather little but the green-manured half yielded a clean crop.

Exp. 2. This was carried out in a garden near Bradford which had been devoted to the growing of a number of potato varieties in 1919. The whole crop was then so badly scabbed that the potatoes were scarcely recognisable. In December 1919, rye was sown thickly over a large strip 57 ft. × 9 ft. across the garden, and the winter being mild, a good crop 10 ins. high was produced by the following May. This was cut with a scythe. The stubble was then dug in, trenches made for planting the potatoes and the green crop strewn in the trenches, little by little, as they were being filled in. For the sake of comparison, another strip was treated with grass at the rate of 15 tons per acre strewn in the trenches as in the case of the rye. This amount of grass had been shown by previous experiments to be an effective dressing for scab. A third strip was planted as a control. No farmyard manure was applied to any of the plots but all were given artificials at the same rate.

The results showed a fair amount of scab on the untreated plot, though this was considerably less than in the previous year. Both the rye and grass plots however gave crops with 90 per cent. clean tubers and no difference between the two plots in this respect could be observed.

Exp. 3. This consisted of a trial of green-manuring on a farm scale. A green crop of rye and vetches 8 to 9 ins. high was ploughed in in the spring of 1920. Within a week of ploughing the land was harrowed and ridged up, most of the green stuff falling into the furrows. Dung was added in the usual way, and the potatoes were then planted. The result of the experiment was, however, negative since both the green manured-part and the control strip which had been left gave clean crops. It is of interest in showing that it is possible on a farming scale to plant potatoes at a very short interval after a green crop has been ploughed in. In this way the decay of the vegetable matter proceeds at the same time as the formation of the new crop, a condition which appears to be essential to the success of the treatment.

The three experiments, all of which were carried out in 1920, suffer from the fact that the amount of scab produced in that year was considerably less than usual, but the results are sufficient to indicate that green-manuring, as carried out by the ploughing in of a growing crop, may be very effective in reducing scab. The writer has since received confirmation of this statement from a well-known firm of seedsmen in this country, who have carried out the treatment with great success, and also from Mr E. Gram of the Experimental Plant Pathology Station, Lyngsby, Denmark, where Lupins were used as the green crop.

Hay and Spent Hops as substitutes for Green-manure.

Exps. 4, 5. The experiments in which these materials were tested were carried out in 1921, which was probably the worst year for common scab on record.

No old hay was available and seeds hay from the previous year's crop was used. This was applied to two small plots at the rates of 2½ tons and 5 tons per acre respectively. Spent hops, in the wet state in which it comes from the brewery, was applied to two other plots at the rates of 5 tons and 10 tons per acre respectively. In each case a control plot was planted. All the plots received artificials but no farmyard manure. The results were as follows:

Treatment (rate per acre)	Description of crop
Seeds hay (2½ tons)	Badly scabbed
„ „ (5 tons)	Moderately scabbed
Untreated plot (1)	Badly „
Spent hops (5 tons)	Moderately „
„ „ (10 tons)	Very slightly „
Untreated plot (2)	Very badly „

It thus appears that seeds hay is of little use as a substitute for green manure and this is possibly due to its stiff coarse nature, on account of which the attack of soil organisms on it is necessarily slow. It is possible that meadow hay being softer in texture would have given better results.

In the case of spent hops, the heavier dressing gave very good results which would certainly justify further trials being made with this otherwise apparently worthless product.

SECTION II. THE EFFECT OF LIME ON SCAB.

The literature on common scab abounds with experiments on liming but the results of these are so conflicting that little can be learnt from them. Similarly, potato growers, in some districts, assert that lime has no effect on scab, whilst others, in different districts, or on soils of a different type, regard liming as the surest way of producing a scabby crop.

There is now little doubt that this apparently inconsistent action of lime is closely connected with the reaction and type of soil to which it is applied. In the experiments which follow, either the initial hydrogen-ion exponent or the lime requirement of the soil or measurements of both data were taken in all cases. The type of soil in Exp. 6 was a very light sandy loam; in Exps. 7, 8, 10, and 11 it was a fairly light silty loam, and in Exp. 9 a similar but somewhat heavier loam.

Exp. 6. This was carried out on a farm near Selby. The principal crop was potatoes and these yielded as much as 16 tons per acre of fine clean tubers on which scab had never been seen. In 1916, however, the farmer complained of the poorness of his rotation crops, in particular of barley and seeds hay, and this was no matter for surprise, when it was observed that the acid indicating weed, spurrey, grew in abundance on the land. A liming experiment was planned and carried out on the field in question by Dr J. A. Hanley, Lecturer in Agricultural Chemistry at this University, to whom I am indebted for the following details. The lime-requirement[1] of the soil was found to be 21 cwts. per acre. Five plots, each one acre in area, were marked out and treated as follows:

No. of plot	Dressing of lime or chalk per acre[2]	
1	Ground chalk (undried)	2 tons
2	,, ,, ,,	5¾ tons
3	Control	
4	Ground quicklime	1 ton
5	,, chalk (dried)	2 tons

In addition, and as a result of the good effect of these dressings on the crops grown from 1916 to 1920, the farmer applied ground chalk at the rate of 5 tons per acre to a further considerable area of the same field. In 1921, the crop was again potatoes and two varieties "Majestic" and "Ally" were grown. At lifting time these were examined by Dr Hanley and the writer, who arrived independently at the same conclusions regarding the amount of scab on the respective plots. These observations, together with the hydrogen-ion exponents of the soils of plots 2, 3 and 4 which were determined on May 19th, 1921, are given in the table below.

No. of plot	*p*H	Degree of scab
1	—	Moderate
2	6·6	Very bad
3	5·0	Practically none. Tubers showing one or two scabs could be found but were not noticeable to a casual observer
4	5·2	Slight
5	—	Rather bad

The "Majestic" crop was perhaps slightly less scabbed than the "Ally," but the relative amount of scab on the different plots was the same in both cases.

On the portion of the field which had been chalked by the farmer the crop was as badly scabbed as on plot 2.

Photographs of the produce of the "Majestic" crop on plots 2 and 3 are shown on Pl. III, Fig. 2.

Exp. 7. This was laid down in the first place as a manurial experiment (which included some liming trials) on pasture land at the University Experimental Farm, Garforth. In 1917, the field was ploughed up and has since carried arable crops, that of 1921 being potatoes. Particulars of the experiment are given in the Guide to the Farm experiments (14), and it is only proposed to state here the details as they concerned the liming of the plots and its effect on the potato crop.

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[1] For the sake of clearness, the lime-requirements given in this paper have been expressed throughout in terms of weight of lime per acre. The figures so given, however, are merely calculated from the percentage of calcium carbonate as determined by the Hutchinson and MacLennan method, and must not be regarded as any exact measure of the amount of lime necessary to bring the soil to a neutral reaction. In practice a quantity of lime considerably in excess of that indicated would probably be required for this purpose.

[2] This chalk contained about 15 % moisture.

Each plot was divided into two parts, a north and a south half.

In 1898, the north half of plot 14 was dressed with quicklime at the rate of 3 tons per acre and the south half at the rate of 6 tons per acre. In addition, in 1911, the north end of all the plots was given ground chalk at the rate of 5 tons per acre. The action of this liming was clearly visible on the soil in 1921, four years after the grass had been ploughed in. On the unlimed portions, the ploughed-in turf lay in undecomposed lumps on the surface after harrowing, whilst, on the limed soil, no evidence of the original turf remained. The potatoes planted were "Great Scot." Observations of the amount of scab on the respective crops were made at lifting time and determinations of the hydrogen-ion exponents for two of the soils—one heavily limed and the other untreated—were made. These data are given in the following table.

Plot		Treatment, rate per acre		*p*H	Degree of scab
14	South	Quicklime	6 tons in 1898	—	Bad
14	North	Quicklime and ground chalk	3 ,, 1898 5 ,, 1911	6·6	Very bad—tubers scarcely distinguishable from the soil
Other plots,	North	Ground chalk,	5 ,, 1911	—	Bad
,,	South	No lime or chalk		4·4	Very little scab—the tubers were spotted to some extent and some showed mature scabs

A photograph of the crops from plot 14 North and from the unlimed plots is shown on Pl. III. Fig. 3.

Exp. 8. This was carried out on a field (No. 112 *S*) at the University Farm in 1915. The lime-requirement of the soil was practically nil. Four plots, each consisting of one row 78 ft. long, were treated as follows:

Plot	Lime applied per acre
1	Untreated
2	5 tons
3	10 tons
4	Untreated

The lime, which was partially slaked, was applied in the rows on top of the dung; a little soil was raked down to cover it and the potatoes then planted.

The crops when examined were found to be all slightly scabbed but no difference whatever could be seen in the relative amount of scab on the four plots.

Exp. 9. This was carried out on a small plot at the University Farm in 1920. The lime requirement of the soil was 9 cwts. per acre, *i.e.* the soil was practically neutral. In this case, it was decided to make a test of magnesian as well as of carboniferous lime, since the former was said to possess a peculiar "burning" effect on root crops. The plot was divided into three parts, which were treated as follows:

Plot	Lime applied per acre
1	Magnesian, 5 tons
2	Untreated
3	Carboniferous, 5 tons

In both cases the lime was partially slaked. It was distributed over the surface of the soil and dug in. Dung was applied in the trenches at the rate of 20 tons per acre and the variety of potato planted was "Great Scot."

All the crops were found to be quite free from scab.

Summary of Experiments 6 9.

The soils on which these experiments were carried out may be divided into two groups according to their reaction before liming. Those of Exps. 6 and 7 were decidedly acid, whilst those of Exps. 8 and 9 were neutral or very nearly so. In the former cases, the application of lime was followed by scab, whilst in the latter, this had no effect. Many similar cases might be quoted and there is little doubt that many of the conflicting statements regarding the results of liming might be reconciled if the initial reaction of the soils treated was known. Superficially, these experiments might be taken to afford full confirmation of Gillespie and Hurst's (2) soil reaction theory for scab, but in the next section, it will be shown that they may be more satisfactorily explained on other grounds. There is no doubt, however, that, on light potato soils, the soil reaction, as measured by its lime-requirement or by its hydrogen-ion concentration may be an extremely useful guide in predicting the action of lime on scab.

The following experiments show that where scab appears on a soil after liming this effect may be counteracted by green manuring.

Exp. 10. This was carried out in 1919 at the University Farm. Two adjacent plots, Nos. IV and V, were chosen on light silty soil. The lime-requirements of the plots were found to be 37 and 38 cwts. per acre respectively. One half of each was given an application of lime at the rate of 50 cwts. per acre, and two days later plot IV was given a dressing of grass cuttings at the rate of 18 tons per acre which was forked in. No farmyard manure was given to either plot, but each received a standard dressing of artificials. Both plots were then planted with "British Queen" potatoes. The results were as follows:

Plot		Appearance of crop
V. Limed half		Scabbed
Unlimed half		Clean
IV. Limed half Unlimed half	green-manured	Clean

A photograph of the crops from plot IV is shown on Pl. IV, Fig. 4.

In the case of plot IV, a few tubers lying at one edge of the plot, where they had escaped the "grass" treatment, showed scab and one of these is included in the photograph on the right hand side. Otherwise, there was no difference in the crops from the limed and unlimed parts of this plot and both showed the clean glossy skins so typical of the green-manurial treatment.

Exp. 11. This was similar to Exp. 10 excepting that a much smaller dressing of grass was used—not more than 2 to 3 tons per acre—on the green-manured plot. The results were almost identical, excepting that the crop treated with lime and grass, although quite free from obvious scabs, showed numerous pin head spots. These were not investigated for want of time, but, in view of the examination of such spots described in Part I (15) of this work, there is little doubt that many of them were very young scabs. This is of interest, since it shows that where the supply of green-manure is too limited or becomes exhausted the potatoes subsequently may be attacked by the scabbing organisms.

A photograph of the spotted tubers from this plot is shown on Pl. IV, Fig. 5.

These experiments prove therefore that where lime exerts a tendency towards the production of scab, this may be entirely counteracted by green-manuring. The significance of this result in its relation to the theories put forward to account for scab is considered in the next section.

SECTION III. AN EXAMINATION OF THE THEORIES ADVANCED TO ACCOUNT FOR THE OCCURRENCE OF SCAB AND ITS PREVENTION BY GREEN-MANURING.

1. *The soil reaction theory.*

This theory doubtless owes its origin to the fact that scab occurs only to a very slight extent in distinctly acid soils. Gillespie and Hurst (2) have now placed this observation on a scientific footing by measurements of the hydrogen-ion concentration of such soils and have further attempted to establish the existence of a close relationship between the hydrogen-ion exponent of *any* soil and the incidence of scab. The conclusions arrived at by these workers are that soils having a hydrogen-ion exponent as low or lower than 5·2 *rarely* produce scab, whilst soils with much higher exponents *generally* produce scab.

We are in agreement with the first part of this statement, although at the same time it should be pointed out that instances of scab in soils of exponents less than 5·2 are not uncommon. Martin (7) has already cited two cases of rather severe scab at exponents of 4·82 and 4·6 respectively, and the present writer mentions the existence of scab in Exp. 7 of this paper, where the exponent was as low as 4·4.

It is, however, with the second part of Gillespie and Hurst's statement that we find ourselves in complete opposition. In the first place we note in the authors' own account of their work that a certain soil "No. 36," having an exponent of 6·22 and falling in the middle of their series of scab-producing soils, yet gave a clean crop of potatoes. This inconsistency was accounted for by supposing that the soil was uncontaminated with scab organisms, since it was "virgin" land. Some years previously, however, Jones and Edson (4) cited a case of scabby potatoes occurring on land which, presumably, had not been in cultivation for 25 years and a part of which had just been cleared of pine. In this case the soil may, of course, have been infected by the use of scabby sets, but it seems much more probable that the Actinomyces capable of producing potato scab are natural inhabitants of the soil.

In any case, however, the argument of "virgin soil" could not be applied in this country, where no kind of control over the planting of scabbed sets has ever been enforced, and where it is impossible that there remain any potato growing soils into which the scab organisms have not at some time or another been artificially introduced. Yet, on thousands of farms clean potato crops are raised and it would be absurd to suppose that in every case the soil was an acid one. Indeed, if we may take such physiological data as the production of a good crop of barley and the absence of acid indicating weeds such as Spurrey, Sheep's Sorrel and Bent on a soil to be a criterion of approximate neutrality, it is an easy matter to recall numbers of such neutral soils, where clean potatoes are regularly grown. In order to verify these observations, it was decided to collect samples of clean potato producing soils from various sources and to determine their hydrogen-ion exponents. The samples were obtained by the courtesy of mycologists, potato growers and gardeners in different parts of England and Scotland and, in each case, the complete or comparative absence of scab is vouched for by the senders. In several cases the land from which the samples were taken was well known to the writer and he is able to confirm the statements regarding absence of scab. The samples were taken to a depth of 6 to 8 inches and at the writer's request, obviously sour soils were avoided.

The values of the hydrogen-ion exponents[1] found are given in the table on p. 78.

Thus, out of 15 soil samples varying from a light sandy loam to heavy clays and including also two peat soils, none showed a hydrogen-ion exponent less than 6·0 whilst the majority gave exponents greater than 7·0. There is no doubt that the number of such neutral or alkaline soils producing clean potatoes could have been added to indefinitely.

These results are obviously a complete contradiction to the conclusions of Gillespie and Hurst who appear to have generalised too quickly from

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[1] In estimating the hydrogen-ion exponents of the soils mentioned in this paper the procedure adopted was as follows:

The air dried soil was powdered as far as possible by finger pressure or by gentle pressure with a wooden block. A portion was then put through a 1 mm. sieve. 20 gm. of this was shaken into a specially cleaned flask with 50 c.c. of neutral distilled water and shaken 75 times. After standing for two days the supernatant liquid was filtered through filter paper, samples of which had been found by previous examination to have no effect on the hydrogen-ion concentration.

The earlier estimations were kindly carried out by Mr N. M. Comber, Lecturer in Agricultural Chemistry at this University, who used the colorimetric method of Gillespie (*Soil Science* 1920, IX, 115); the later estimations were made by the writer using the older colorimetric method of Clark and Lubs (*Journ. Bact.* II, Nos. 1, 2, 3). Comparative tests by Mr Comber and myself showed that the two methods gave closely approximating results.

Source	Type of soil	*p*H	Remarks
1. Nostell, Yorkshire	Medium loam	7·7	Sent by a head gardener who had never known scab to occur on it. The 1921 crop was examined by the writer and found to be perfectly clean
2. Doncaster, Yorkshire	Light loam	7·7	No scab known to occur on this soil before 1921 when the crop was very slightly scabbed
3. Wedmore, Somerset	Medium loam	7·9	From the writer's own garden where scab is unknown
4. ,, ,,	,, ,,	7·6	Scab unknown
5. ,, ,,	,, ,,	7·8	,, ,,
6. Sand, Somerset	Sandy loam	7·4	Ploughed out grass land in 1917. Subsequent crops free from scab
7. ,, ,,	Heavy loam	7·8	Scab very rare
8. ,, ,,	Heavy clay	7·6	,, ,, ,,
9. Wellington, Somerset	Sandy loam	7·7	,, unknown
10. Settle, Yorkshire	Light loam	6·1	These soils overlie limestone. Crops invariably clean
11. ,, ,,	,, ,,	6·0	
12. Moorland, Somerset	Peat	7·1	Clean crops always produced
13. Fen district, Cambridgeshire	,,	7·6	,, ,,
14. Innerwick, Dunbar, Scotland	Red Sandstone	7·1	The clean potatoes grown in this district are well known
15. ,, ,,	,, ,,	7·1	

their work on the Caribou and Washburn soil types. It becomes apparent, therefore, that although a high hydrogen-ion concentration may serve as an indication of the absence of scab in a soil, the converse statement is by no means true.

Some neutral and alkaline soils produce clean crops and others produce scabbed crops, and the reaction of the soil cannot, therefore, be regarded as the determining factor in the incidence of scab.

2. *The preferential food theory.*

This theory was advanced by the writer in an earlier report (8). It was there named the "Decoy" theory, but may here be more accurately described under the name given above. It is based on the established fact that when a sufficient quantity of green vegetable matter is introduced into a scabbing soil, clean potato crops may be grown and a practical point on which some stress has been laid is that the more intimately the soil and green-manure are mixed, the better are the results of the treatment.

Adherents of the soil reaction theory have naturally attempted to explain these results by suggesting that the inhibitory effect of the green-manure was due to a rise in the hydrogen-ion concentration of the soil brought about by its decay. Stephenson (10) has shown, however, that vegetable matter such as Rape and Soy Beans, when applied in a dry state at the rate of 10 tons per acre actually reduced the hydrogen-ion concentration. A few tests were made by the writer during the summer of 1921 on some green-manured and untreated soils which had been ridged up for potatoes in the ordinary way. The green-manure had been applied as short grass at the rate of 20 tons per acre. Estimations of the hydrogen-ion exponents of the green-manured and untreated soil ridges gave the following results:

	*p*H values	
Date 1921	Green-manured soil	Untreated soil
May 25	4·7	4·5
July 28	4·8	4·4
Aug. 15	4·8	4·4
Oct. 14	4·9	5·0

It should be pointed out that the samples taken were from ridges 6 ft. long and composite samples were not made from the whole of the ridge. This may possibly account for the curious increase in the exponent for the untreated soil between the two last dates on which samples were taken.

In the case of the green-manured soil, however, the figures show a gradual decrease in the hydrogen-ion concentration and thus agree with Stephenson's results.

It appears impossible therefore to account for the effect of green-manuring by the soil reaction theory.

The preferential food theory makes the assumption that *Actinomyces scabies* is primarily a saprophytic organism living on organic remains in the soil and showing a partiality for the more undecomposed parts of vegetable matter. Only when the supply of such food stuff is deficient does it attack the potato tubers.

Shapovalov (9) has already called attention to the saprophytic nature of the organism and its "parasitic possibilities."

The writer has found that sterilised grass cuttings make the finest medium known for rapid growth of strains of *A. scabies* and that sterilised samples of the peat soil from Somerset mentioned on p. 78 also permitted good growth. Photographs of the cultures made on these two media may be seen in the Report (8) mentioned.

On the assumption made, it is easy to understand the scab-inhibiting effect of green-manure and the necessity for a close admixture of the green stuff with the soil, but the theory receives its strongest support from the remarkable way in which it accounts for the established facts concerning scab. These may be considered as follows:

(1) True peat soils irrespective of their hydrogen-ion concentration are comparatively free from scab, and this may now be explained by the fact that, in such soils, the large natural reserve of organic matter serves as a constant food supply to the Actinomyces present. In a recent review of the writer's Decoy theory, Drayton (1) states that he was unable to find any Actinomyces in certain soils, principally peat, or peat and clay, which were of high hydrogen-ion concentration. It will be remarked however that the maximum count given by this author in an examination of 23 soils is less than one million per gram, which is a very low figure. Although, therefore, without any standardised method of making Actinomyces counts, the relative figures of different investigators are of little value, we suspect that Drayton's technique is at fault in finding soils totally devoid of these organisms.

The present writer found as many as 700,000 Actinomyces per gram of the soil mentioned in Exp. 7 which had a hydrogen-ion exponent of 4·4 and on which slight scabbing occurred. The counts for neutral or alkaline soils are however generally much higher and the writer does not question the statement that acidity depresses the Actinomyces flora, and thus reduces the likelihood of scab. This does not in any way vitiate the application of the preferential food theory to peat soils. Thus, the Actinomyces count for the Somerset peat soil mentioned on p. 78 and having a hydrogen-ion exponent of 7·1 was 5,000,000 per gram. Yet, this soil yields perfectly clean crops each year.

(2) Scab is most prevalent and most virulent on light sandy or gravelly soils. The well aerated condition of such soils is peculiarly suited to the development of the *A. scabies* group, and probably of the majority of the genus. Bacterial action is also rapid and thus manure, whether farmyard or vegetable, tends to disappear quickly. The condition thus brought about in such soils is the reverse of that in a peat soil and scabbing ensues. Clean potatoes may sometimes be obtained on such soils by early lifting. It is on land of this type that heavy green-manuring is particularly efficacious.

(3) Lime produces or aggravates scab under certain conditions.

From the experiments described in Section II the following facts stand out:

(*a*) The presence or absence of scab on neutral soils is unchanged by liming.

(*b*) The addition of lime to a distinctly acid soil tends to produce or increase scabbing.

(*c*) This deleterious effect may be counteracted, if heavy dressings of green-manure are applied to the limed soil.

As already pointed out (*a*) and (*b*) if considered alone might be taken as confirmation of the soil reaction theory. A point in connection with liming results is however generally overlooked. According to the soil reaction theory, we have to assume that soils of a certain degree of acidity are void of scab organisms. When, however, such soils are limed, intensely scabby crops may be produced and we may well ask from what source the scab organisms appear in such numbers as to produce this effect.

When next we consider (*a*), (*b*) and (*c*) together, it will be seen that the whole case cannot be explained by the change in soil reaction, since it has already been shown that green-manuring, though exerting only a slight effect on the soil reaction, tends to move it in the same direction as lime.

The facts are however easily accounted for by the Preferential food theory. Lime favours the development of the Actinomyces both by neutralising acidity and by opening the soil. At the same time, it hastens the decay of plant residues in the soil. Thus, on the one hand, the scab organisms are multiplied and, on the other, their natural food supply dwindles. The potatoes are then attacked. If, however, the deficiency in the food supply is made good by the addition of green stuff, the tubers remain free from infection. In the case of (*a*), it is obvious that, where no deficiency of lime exists, a further addition will produce no ill effect. In this case, if the soil is well supplied with vegetable matter in a palatable state for the scab organisms, the crops will be clean, and, if it is not, they will be scabbed.

Some critics of this theory have urged that the green matter introduced, if so favourable a medium for Actinomyces, would be quickly smothered with these organisms and disappear in a very short time. This, however, is not the case, since Actinomycetal action at soil temperature is much less rapid than at the higher temperatures adopted in artificial cultures. In fact, in the green-manuring experiments described, which were carried out on light dry soils, a certain amount of the grass added was invariably seen when the potatoes were lifted.

Others, whilst accepting the idea of the green organic matter as a preferential food have raised the objection that the spores of the Actinomyces will naturally germinate *in situ* and, in some cases, this must be on the potato itself. We are bound to admit that this objection seems very sound. It should, however, be remembered that spore formation in *A. scabies* is very variable. In cultures, it may often be retarded indefinitely by an abundance of food; it is also much checked by an acid reaction of the medium, and by low temperatures. Again, the power to produce an aerial mycelium with spore formation is often entirely lost by continuous cultivation of the fungus on the same medium. To what extent these various factors affect spore formation of the fungus in a soil highly enriched with vegetable matter, we are not prepared to say. It is an interesting fact, however, that on potato scabs themselves, the aerial mycelium bearing the spores of the fungus is very scanty.

There is lastly the possibility that the latent parasitic power in the scab organisms is developed only under the stress of hunger and thus, spores or fragments of mycelium from a parent mycelium growing luxuriantly on a saprophytic diet are powerless to attack the potato tuber. In 1921, an attempt was made to test this point by inoculating growing potato tubers with a strain of *A. scabies* previously cultured on sterilised grass cuttings for six generations of one week each. In some cases, the skin of the potato was first scratched with a sterile needle and the inoculum rubbed on the scratch with a platinum loop, whilst, in others, drops of the culture emulsion were spotted on to the tubers. In all, 10 tubers were inoculated. Seven of these showed no signs of infection whilst the remaining three showed only two or three very small scabs. Thus, whilst it would be unwise to draw definite conclusions from a single test, the results certainly suggest a loss of parasitic power in the organisms following a generous saprophytic diet.

There does not appear therefore to be any insuperable objection to the Preferential food theory, which may not be removed by an extended knowledge of the morphological and physiological characters of the Actinomyces group.

3. *Soil moisture and soil temperature as factors in scab production.*

Observations extending over a number of years have shown that common scab is much more prevalent and occurs with far greater virulence in dry than in wet seasons. An illustration of this is afforded by recent years. Thus in 1919 and 1921, the growing season was exceptionally dry and the disease very severe, whereas in 1920, which was very wet, little scab appeared.

In this country, dry summers are usually accompanied by heat and wet summers with cold. Thus, in the action of climatic conditions on the disease, two factors, soil temperature and soil moisture, either or both of which may play an important part, must be considered. In America, L. R. Jones and McKinney (5, 6) have attempted to establish a relationship between soil temperature and scab development. They point out that scab is more prevalent in the southern and warmer regions than in the northern and cooler States. They have not so far given any figures for the temperatures of these soils, but have shown by greenhouse experiments that the optimum temperature for the development of the scab organism in the soil is about 24° C. The geographical evidence in this country is scarcely in support of these observations since scab is, if anything, more general and severe in the north of England than in the south. In fact, the disease appears to have been a serious problem to farmers in Yorkshire long before it was given any attention at all in the south of England.

Following on the hypothesis of Jones and McKinney, a suggestion was made to the writer by Dr Pethybridge of the Royal College of Science and Technical Instruction for Ireland that the beneficial effect of green-manuring for scab might be due to a reduction of temperature in the treated soil such as would result from a mulch. It was decided therefore to carry out experiments to ascertain (1) what reduction of temperature was actually brought about by mulching, (2) the effect, if any, which such a reduction produced on scabbing, and (3) the reduction of temperature, if any, brought about by a green-manuring carried out on the lines of the previous experiments.

An account of the experiments (Nos. 12 and 13) is given below.

Exp. 12. This was carried out in 1921. A plot 22 ft. × 6 ft. was chosen on soil of a scabbing nature. Its lime-requirement was found to be 33 cwts. per acre and a dressing of lime was applied at the rate of 2½ tons per acre. Later, the plot was dunged at the rate of 15 tons per acre and planted with "Great Scot" potatoes. It was then divided into three parts, *A*, *B* and *C*. In order to carry out the mulching as effectively as possible, the potatoes were not earthed up on any part of the plot. When the tops were about 6 ins. above the ground, *A* and *C* were given heavy mulches about 3 ins. thick of grass cuttings and farmyard manure respectively, whilst *B* was left untreated. A thermometer enclosed in lead piping was then inserted to a depth of 6 ins. between two plants in a row in each of the three plots. Eleven readings were taken at intervals throughout the season from June 18 to October 12 and were as follows:

Date	Temperature ° C.		
1921	*A* (Grass mulch)	*B* (Untreated)	*C* (Manure mulch)
June 18	16·0	18·5	16·0
„ 25	19·0	21·5	18·5
„ 28	17·0	20·0	18·0
July 6	16·0	17·0	16·0
„ 14	18·0	20·0	19·0
„ 26	18·5	20·0	18·5
Aug. 3 (after rain)	15·0	15·5	15·5
„ 15 „ „	15·0	16·0	15·5
„ 22	17·5	18·0	17·25
„ 27	16·0	16·5	17·0
Oct. 12	14·5	15·0	14·5
Average of readings	16·6	18·0	16·9

From the above it will be seen that the reduction in temperature effected by the grass and dung mulchings was practically the same and that this amounted only to an average of 1·25° C.

A good crop of potatoes was lifted in spite of being grown on the flat and the results as regards scab were as follows:

Part *A* (grass mulched)	Only moderately scabbed.
,, *B*, untreated	Badly scabbed.
,, *C* (dung mulched)	,, ,,

No difference whatever between *B* and *C* was apparent, so that obviously the reduction of temperature effected on plot *C* had exerted no retarding action on the scabbing and the diminished amount of scab of plot *A* cannot therefore be ascribed to this cause. It may, however, be accounted for by an observation made on lifting the crop, namely, that a certain amount of the fine grass used had partially rotted and become incorporated with the uppermost layer of the soil. Thus, since the crop lay very close to the surface of the soil, a large proportion of the tubers were growing in a mixture of soil and grass such as they would get in a green-manurial treatment carried out in the ordinary way.

Exp. 13. On a second plot, a green-manurial experiment was put down on half of which grass cuttings were applied and forked in at the rate of 20 tons per acre. Thermometers were inserted in one ridge of the "grassed" soil and one ridge of untreated soil adjacent to each other and readings taken at intervals. These were as follows:

Date 1921	Temperature ° C.	
	"Grassed" soil	Untreated soil
July 14	22·0	23·0
,, 25	22·0	22·5
Aug. 3	17·5	18·5
,, 15	19·0	20·5
,, 22	19·25	21·0
,, 27	17·0	18·0
Sept. 28	15·5	16·5
Oct. 12	15·0	16·0
Average reading	18·4	19·5

The average reduction of temperature therefore due to the admixture of the grass is just over 1° C. and in view of the results of Exp. 12, we must conclude that this reduction can have no appreciable effect in inhibiting scab. In any case, it would be scarcely possible to suppose that so small a difference in temperature would retard the growth of the scab organism to more than a very slight degree. The temperatures noted in the last table certainly approach the optimum of 24° C. given by Jones and McKinney (6) for the scab organism and in such a summer as that of 1921 it is more than probable that the high soil temperature would favour the development of these organisms. On the other hand, however, soil temperatures taken in 1914, on a plot which produced a very scabby crop were considerably lower. Thus, 12 readings taken at 11 a.m. at intervals from July 28 to August 17 gave an average temperature of

13·2° C. and a maximum temperature of 14·3° C., whilst 12 readings taken at 1 p.m. at intervals from August 18 to September 2 gave an average temperature of 16·0° C. and a maximum of 18° C. Such temperatures are much the more normal for this part of the country, but they fall considerably below the optimum for *A. scabies.*

Whilst admitting, therefore, that, provided other conditions are favourable to the disease, a high soil temperature will favour its development, we do not think that this factor can exert more than a secondary rôle in the incidence of scab. It appears far more probable that the moisture content of the soil is the seasonal factor of greater importance.

Soil moisture. In the report (8) on Common Scab already referred to, experiments were described which led us to abandon the hypothesis of the moisture content of the soil being the factor directly responsible for the presence or absence of scab on any given soil and the factor which appeared to us to be fundamental in scab control has already been discussed in this section.

Nevertheless, on soils already favourable to scab, the water content appears to exert an indirect but often powerful influence. It should first be pointed out that the climatic conditions affecting scab only do so when they are very pronounced. Thus, a very dry year brings much scab and a very wet year little, but a moderately wet or moderately dry year has little effect one way or the other.

It is well known that dryness means high soil aeration especially of the surface layers of the soil and excessive wetness means that, except on extremely light soils, the pores of the soil will be constantly choked with water and aeration will be poor.

Previous workers on the Actinomyces group have shown that these organisms are very sensitive to the air supply. Waksman (13) states that out of a large number of species isolated from the soil none grew under strictly anaerobic conditions. Similarly, Mr S. Burr, Demonstrator in Agricultural Botany at this University, has found that none of 12 strains of *A. scabies* examined gave any growth under strictly anaerobic conditions. Most of the strains were able to make some growth under partially anaerobic conditions, as, for example, at the bottom of a liquid culture, but this was greatly increased with a further supply of air. It may safely be assumed therefore that the members of the *A. scabies* group are preferential aerobes. They are, moreover, extremely resistant to drought and cultures which were allowed to dry up have been found viable after a period of two years. Many strains, indeed, produce their spores most readily in culture when the medium begins to dry up.

Thus, it would seem that the continuously high aeration of the soil in a dry year is particularly favourable to the development of the scab organisms, and, in the light of this conclusion, it is possible to explain two facts regarding the incidence of scab which seem incapable of explanation in any other way. Thus (1) certain very gravelly or sandy soils are so totally unaffected by climatic conditions that they produce badly scabbed crops in all seasons. An instance of this type of soil may be found in an account of potato scab by Seton and Stewart(12) and the soil there referred to is well known to the writer. Such soils are so porous that they are never clogged with water even in the wettest seasons and the Actinomyces in them do not suffer at any time from lack of air. (2) Clay soils are far less liable to produce scab than lighter soils. Here, again, this partial immunity to scab would seem to be closely associated with the restricted air supply which is at a minimum in a heavy soil. Soil aeration therefore appears to be the dominant factor in the influence exerted by dry and wet seasons on scab.

SUMMARY OF PARTS I AND II.

1. Common Scab consists of a number of types of scab, which vary considerably in general appearance. Of these, the two most marked types have been called "Raised" and "Pitted" respectively. Other types are intermediate between these extremes.

The causal organism in all cases examined belongs to the Actinomyces genus. The different strains isolated exhibit considerable differences in culture but, for the present, they may be placed in a single Actinomyces scabies group.

2. The incidence of scab is closely associated with soil type. It occurs most commonly and with greatest virulence on light, sandy or gravelly soils, especially on those of a "hungry" nature. Conversely, it is rarely found on peat soils.

3. The disease may be inhibited by the application of sufficiently liberal dressings of green-manure to the soil. Spent hops have also proved to be of value in this respect and leaf mould has long been used by gardeners with good results.

4. The action of lime or chalk on scab production depends largely upon the initial reaction of the soil. On neutral soils, it exerts little or no effect. On distinctly acid soils, it tends to produce or aggravate the disease unless the soil contains a large reserve of vegetable organic matter. This effect may be counteracted by green-manuring.

5. The hydrogen-ion concentration of the soil is not the direct factor

of control in the occurrence of scab and this statement is borne out by the following facts:

(*a*) Scab is found to only a slight extent in soils of a high hydrogen-ion concentration, yet, it *may* occur and Actinomyces have been found in large numbers in soils with a *p*H value as low as 4·4.

(*b*) In more nearly neutral soils, there is no relation between the hydrogen-ion exponent of the soil and the occurrence of scab.

Thus, for example, one soil having a *p*H value of 7·0 may yield absolutely clean crops, whilst another with the same exponent may produce very scabby crops. This distinction cannot be explained by supposing the former soil to be virgin and uncontaminated with scab organisms.

(*c*) In the treatment of scab by green-manuring, any change brought about in the soil reaction appears to be towards a decrease rather than an increase in the hydrogen-ion concentration.

6. The established facts concerning the common occurrence of scab on light soils, its comparative absence on peat soils, the action of lime and the cure of the disease by green-manuring may be explained by the Preferential food hypothesis. According to this theory, the scab organisms are primarily saprophytic living on vegetable remains in the soil. They remain so until their natural food supply is exhausted and develop their parasitic tendencies only under the stress of hunger.

7. Scab is more prevalent in dry than in wet seasons. This is no doubt due in some small degree to the higher soil temperature obtaining in dry seasons. The climatic factor of greatest importance, however, would seem to be rainfall which modifies the air content of the soil and thus regulates the development of the scab organisms which are strongly aerobic. The effect is most marked in clay soils where scab is almost entirely inhibited by a wet season.

The writer wishes to express his sincere thanks to the following colleagues: Mr F. T. Bennett, Mr S. Burr, Mr N. M. Comber, Dr J. A. Hanley, Mr J. Manby, Professor Seton and Miss L. Scott.

EXPLANATION OF PLATES III AND IV

Fig. 1. Effect of green-manuring on scab. Left: green-manured. Right: untreated. (Reproduced from *Report* No. 118, University of Leeds and Yorkshire Council for Agricultural Education.)

Fig. 2. The effect of liming on scab. Experiment 6. Left: chalked. Right: untreated.

Fig. 3. The effect of liming on scab. Experiment 7. Left: limed and chalked. Right: untreated.

Fig. 4. The effect of lime counteracted by green-manuring. Experiment 10. Left: green-manured but unlimed. Right: green-manured and limed.

Fig. 5. The effect of lime partially counteracted by green-manuring. Experiment 11. Note the pin point scabs on all the potatoes.

Note. The blocks for the above plates have been kindly lent by the University of Leeds and the Yorkshire Council for Agricultural Education.

REFERENCES.

(1) DRAYTON, F. L. (1921). *Int. Report, Division of Botany, Dept. of Agric. Canada*, 17.
(2) GILLESPIE and HURST (1918). *Soil Sci.* VI, 229–35.
(3) GILLESPIE, L. J. (1918). *Phytopathology,* VIII, 6, 257–68.
(4) JONES, L. R. and EDSON (1901). *Vt. Agri. Exp. Report,* XIV, 231–5.
(5) JONES, L. R. and MCKINNEY (1919). *Phytopathology,* IX, 7, 301–2.
(6) —— —— (1920). *Ibid.* X, 1, 63.
(7) MARTIN, W H. (1920). *Soil Sci.* IX, 393–407.
(8) MILLARD, W. A. (1920). *Report* 118. Univ. of Leeds and Yorks. Council for Agric. Educ. 1–22.
(9) SHAPOVALOV, M. (1919). *Phytopathology,* IX, 9, 422–4.
(10) STEPHENSON, R. E. (1921). *Soil Sci.* XII, 2, 145–62.
(11) STEWART, J. G. (1906). *Report* 63. Univ. of Leeds and Yorks. Council for Agric. Educ. 22.
(12) —— (1907). *Report* 70. Univ. of Leeds and Yorks. Council for Agric. Educ. 15.
(13) WAKSMAN, S. A. (1919). *Soil Sci.* VIII. 83.
(14) Guide to Experiments at Manor Farm, Garforth (1921). *Report* 120. Univ. of Leeds and Yorks. Council for Agric. Educ. 18.
(15) MILLARD, W. A. (1922). *Ann. App. Biol.* IX, 2.

(*Received May* 29*th,* 1922.)

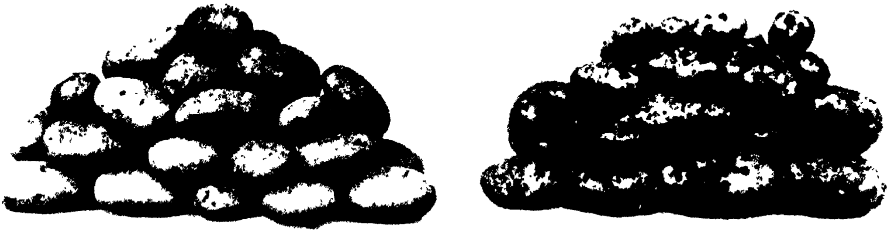

Fig. 1.

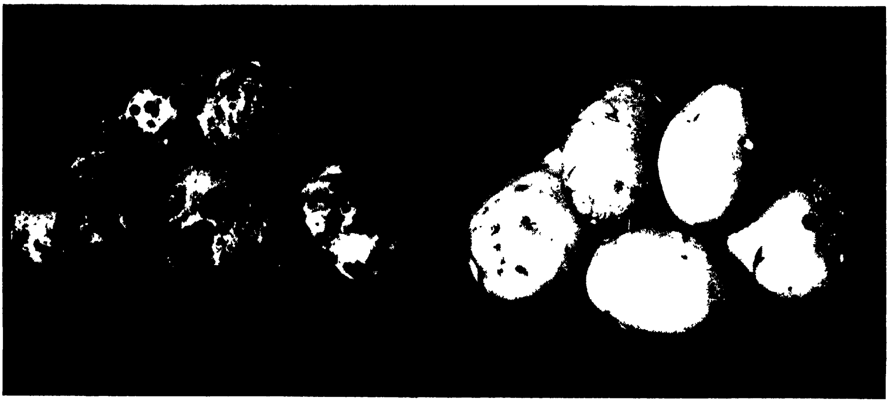

Fig. 2.

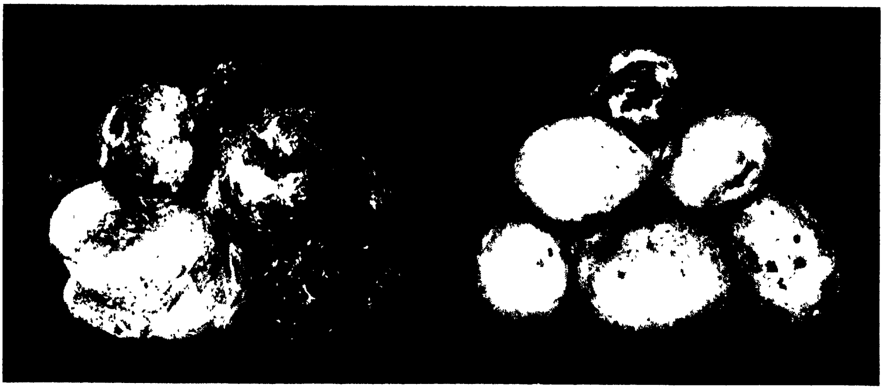

Fig. 3.

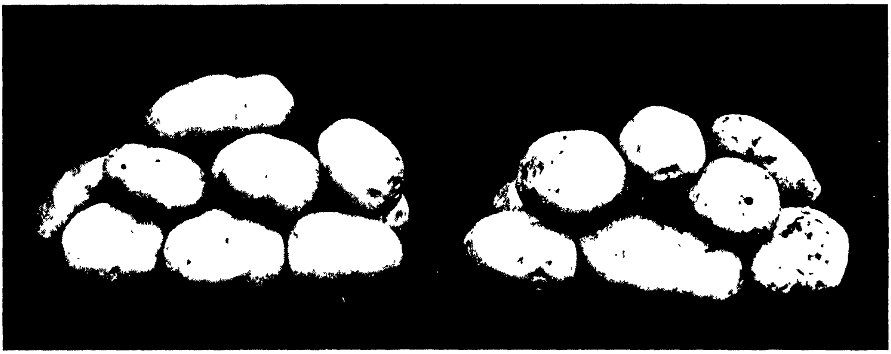

Fig. 1

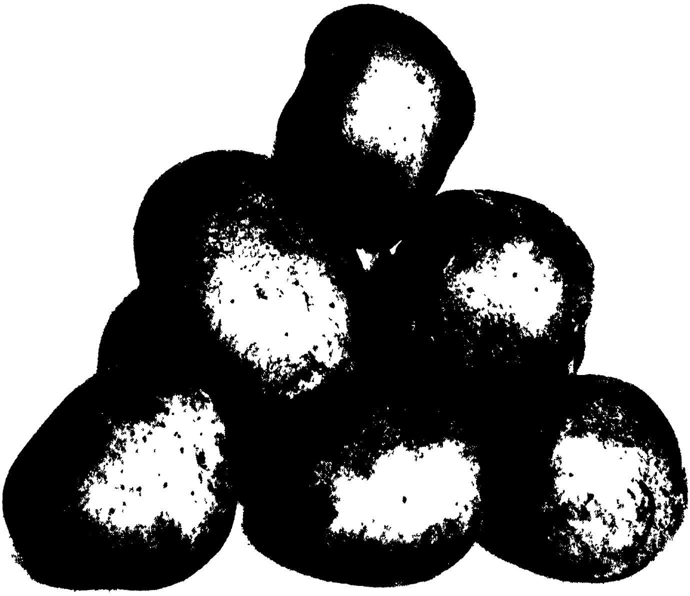

Fig. 5.

STUDIES IN BACTERIOSIS. VIII

FURTHER INVESTIGATION OF THE "STRIPE" DISEASE OF TOMATO

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In a previous communication (2) the results of an investigation upon "Stripe" disease of the tomato were described. The isolation of the causal organism, a yellow bacillus, and its resemblance to *B. lathyri*, Manns and Taubenhaus (1) was reported. Owing to the fact that the identity of the organism was not discovered until after the paper had been sent to the press, it was not possible to publish precise proof of identity, namely the cross-inoculability of the "Stripe" organism from the tomato to the sweet pea. The present paper is concerned with the results of a series of cross-inoculation experiments of the "Stripe" organism from the tomato into a number of other plants, chiefly legumes.

EXPERIMENTAL.

Bacillus lathyri was again isolated from typical "Stripe" lesions on tomato stems and tested for pathogenicity by pricking a small piece of a pure culture, under aseptic conditions, into rapidly growing young tomato plants, when the typical lesions of the disease were readily produced. Inoculations with the organism were then performed upon a large variety of plants. This was done in three ways; by seed inoculation, by stab inoculation and by spraying the plants with a suspension of the organism. The plants showed "soft" growth as a result of liberal dressings of a fertiliser containing a high percentage of nitrogen; the best conditions for the disease were thus provided. *Seed inoculation* gave positive results in a limited number of species, but the stab and spray inoculations indicated that a large number of plants are susceptible to the organism.

Stab inoculations. Small pieces of a pure culture were pricked into the base of the stem, under aseptic conditions, and the wound covered with tinfoil. The external lesions were some time in appearing, usually from four to seven weeks, but on opening the stem with a knife in the region of the point of inoculation, the destruction of the pith could be seen within a week of the inoculation.

Culinary Pea (*Pisum sativum*).

17. iii. 20. Inoculated.
4. v. 20. Small brownish black sunken spots and thin streaks appeared on the stem up to five inches above the stab.
26. v. 20. Lesions showed an increase in size and number and had appeared on the leaves.

Control plants (pricked with a sterile needle) were quite healthy.

Lupin (*Lupinus* sp.).

17. iii. 20. Inoculated.
29. iv. 20. Thin brown streaks on the stem just above the stab.
6. v. 20. Leaves showed many minute sunken black spots.
26. v. 20. Stem showed numerous purple brown streaks and blotches, while the affected leaflets were turning yellow and many had fallen off the petiole.

Control plants were quite healthy.

Red Clover (*Trifolium sativum*).

17. iii. 20. Inoculated.
20. iv. 20. Long light brown sunken streaks appeared on the inner side of the petioles.
26. v. 20. Six petioles per plant showed lesions; the leaflets were covered with small brown spots and patches.

Controls were healthy.

Sainfoin (*Onobrychis sativa*).

17. iii. 20. Inoculated.
19. iv. 20. Thin black sunken streaks appeared on the inner sides of the petioles.
26. v. 20. Many petioles were smothered with lesions, while the leaflets showed black sunken spots.

Control plants were quite healthy.

Potato (*Solanum tuberosum*).

Potato tubers, variety Epicure, were planted in loam in six inch pots and when about four inches high were inoculated.

14. vii. 20. Inoculated.
27. viii. 20. Light brown sunken furrows appeared on the shoots above the point of inoculation.
5. ix. 20. Lesions on the stem were more numerous and larger; some furrows being three centimetres long and three millimetres broad.
10. ix. 20. Inoculated plants had turned yellow in those parts of the stem where the lesions were most pronounced, but there were no lesions upon the leaves.

Controls were quite healthy.

Seed inoculations. Seeds were sterilised in mercuric chloride, washed in sterile water and inoculated by soaking for fifteen minutes in a water suspension of the bacilli. After drying, they were planted in sterilised soil. Seeds treated in a similar manner, but soaked for fifteen minutes in sterile water instead of the bacterial suspension, served as controls. After six weeks, the culinary pea and red clover were the only species to show signs of the disease.

Spray inoculations. For the purpose of this series of experiments the bacterial suspensions were prepared in two ways as follows:

(1) Twenty-five c.c. flasks with 15 c.c. of beef bouillon each were sterilised and inoculated from a beef agar slant culture. After five days' incubation at 22° C. each 15 c.c. were diluted to 500 c.c. by the addition of sterile water at 22° C.

(2) Ten c.c. of sterile distilled water at 22° C. was added to a five days' culture on beef agar and the bacterial mass scraped off by means of a platinum needle. The bacterial suspension was poured from the tube and made up to 500 c.c. by the addition of sterile water. Six plants of each kind were sprayed by means of a small hand spray with suspension (1) and six with suspension (2). Six plants were sprayed with sterile water as controls. Precisely similar results were obtained with each suspension.

Culinary Pea (*Pisum sativum*).

7. vi. 20. Inoculated.

11. vi. 20. Numerous lesions appeared as tiny sunken black spots on leaves, tendrils, pods and stems.

14. vi. 20. The leaves were abundantly spotted; the spots running together to form larger patches and the leaves beginning to wither. The lesions on tendrils and stems showed as numerous blackish brown sunken furrows, four to seven millimetres in length. The pods showed dark brown sunken spots and patches.

20 vi. 20. The plants were smothered in lesions, which in places were two to three millimetres wide. Many leaves were completely withered. The organism was re-isolated from the lesions.

Control plants were quite healthy.

Red Clover (*Trifolium sativum*).

7. vi. 20. Inoculated.

16. vi. 20. Tiny light brown spots appeared on leaflets and petioles.

20. vi. 20. Long light brown sunken furrows on petioles. Leaflets spotted and withering. The organism was re-isolated from typical lesions.

Control plants were quite healthy.

Broad Bean (*Vicia faba*).

7. vi. 20. Inoculated.

16. vi. 20. Tiny dark brown spots showed on the leaflets and stem.

20. vi. 20. Leaflets well spotted with dark brown sunken spots; several withering. Thin purplish brown streaks three to six millimetres in length showed on the stem. The organism was re-isolated from the lesions.

Control plants were healthy.

French Bean (*Phaseolus vulgaris*).

7. vi. 20. Inoculated.

14. vi. 20. Light brown spots on leaves and light brown patches and furrows on stem, petioles and leaf veins.

20. vi. 20. Lesions fairly numerous, but not so far advanced as the other species. The organism was re-isolated with difficulty.

Controls quite clean and unspotted.

Sweet Pea (*Lathyrus odoratus*).

7. vi. 20. Inoculated.

14. vi. 20. Light brown spots on leaves. Tissues yellowing round the spots. Light brown furrows, sunken and splitting, on leaf petioles.

20. vi. 20. Lesions more numerous and intense. Stem, petioles, leaves and flowers all attacked. Organism re-isolated.

Controls healthy.

Lucerne (*Medicago sativa*).

7. vi. 20. Inoculated.

18. vi. 20. Small brown sunken spots on the leaves and similar streaks on the stem.

20 vi. 20. Spots and streaks larger and more numerous. Organism re-isolated.

Controls quite healthy.

Lupin (*Lupinus* sp.).

7. vi. 20. Inoculated.

16. vi. 20. Small sunken black spots on the leaflets and light brown streaks on the stem.

20. vi. 20. Many leaflets covered with lesions. Spots larger and concurrent. Leaflets turning yellow and falling. Stem covered with dark brown lesions, which are splitting longitudinally. Organism re-isolated.

Controls healthy.

Vetch (*Vicia sativa*).

7. vi. 20. Inoculated.

16. vi. 20. Many small light brown to reddish purple spots on leaflets and petioles.

20. vi. 20. Some leaflets almost completely covered with lesions. Leaflets yellowing and withering. Organism re-isolated.

Controls healthy.

Sainfoin (*Onobrychis sativa*).

7. vi. 20. Inoculated.

15. vi. 20. Numerous black spots on leaflets and petioles.

20. vi. 20. Many leaves covered with lesions, turning yellow in places and withering. Petioles covered with short black sunken furrows two to four millimetres in length. Organism re-isolated.

Controls healthy.

Negative results were obtained with *Melilotus*, *Ulex* and *Lotus corniculatus*.

The re-isolations of the organism from diseased plants in the above experiments were grown for five days upon potato-mush-agar and when pricked into young rapidly growing tomato plants readily produced the typical "Stripe" lesions. Their cultural reactions also showed them to be identical with the organism employed in the original inoculations.

It will be seen, therefore, that the organism which causes "Stripe" disease of tomato has a wide range of host plants, a fact which is of great importance to the commercial grower. Many cases are recorded where tomatoes grown in houses built upon pasture, clover leys etc., have been badly attacked by "Stripe" in the first year. This has been in the past a puzzle to the practical man, who naturally expected a clean healthy crop on the new soil. Careful investigation has shown that whenever "Stripe" occurred in the first year, the preceding crop had included legumes. In cases brought to the personal notice of one of us (W. F. B.) the previous crop was clover, lucerne and field peas respectively.

Again those parts of the houses, which have been occupied by hedges in the previous year are frequently to be distinguished by the position of "striped" plants, which mark the spots where vetches and other leguminous weeds grew previously. The whole of the evidence emphasises the necessity for removing all weeds from the vicinity of nurseries. Growers who contemplate building nurseries upon fresh land, would do well to have the land examined by an expert to ascertain if the existing crop is free from "Stripe" disease.

DISEASE TRANSMISSION BY TOMATO SEED.

It has long been suspected that "Stripe" of tomatoes is sometimes transmitted by the seed. While definite scientific proof has not so far been forthcoming, observations on commercial nurseries indicate that this is extremely probable. One case in particular was very suggestive. Until 1920 this nurseryman had grown tomatoes for over 14 years without "Stripe," but this year he bought a little seed to make up a shortage. Plants raised from the bought seed developed "Stripe" and were the only plants out of a considerable number that were so affected. Similar cases have been repeatedly brought to the junior author's notice, and so vital does the problem appear that experiments were set up to investigate it.

Two sets of seed were used; one obtained from a badly diseased fruit and another from an artificially inoculated fruit. The seeds were carefully extracted and dried in 1919 and sown in sterilised soil the following year. Sterilised seed-boxes, pots and water were also used. The plants raised from the naturally infected fruit showed two out of sixteen with "Stripe" and those from the inoculated fruit showed one out of sixteen. Such results are far from being conclusive but they are probably more instructive than at first appears. They were the only plants out of two hundred in the house that developed "Stripe." Microtome sections have been made of seeds from diseased and inoculated fruits and in some cases the bacilli have been found under the testa. This rather indicates that the bacilli may sometimes be carried in this way, but there is always the possibility that seeds attacked by the organism may be destroyed by them and so prevented from transmitting the disease.

THE EFFECT OF DIFFERENT MANURIAL TREATMENTS ON THE INCIDENCE OF THE DISEASE.

The yearly observations begun last year in the experimental houses at Cheshunt were continued in 1920. They confirmed those of the previous year, *i.e.* that plants showing hard growth produced by suitable dressings of potash are more resistant to "Stripe" than those showing soft growth resulting from excess of nitrogen unbalanced by suitable amounts of potash.

			No. of diseased plants	
Variety	Treatment	Total No. of plants	1919	1920
Comet	C.A. without potash	120	78	105
,,	Control untreated	120	50	68
,,	C.A. with dung	120	45	50
,,	C.A. without phosphates	120	41	51
,,	C.A.	120	40	41
,,	C.A. without nitrogen	120	34	30
,,	Double C.A.	120	34	34
Kondine Red	C.A. without potash	120	33	96
,, ,,	C A. with dung	120	33	38
,, ,,	Control untreated	120	30	48
,, ,,	C.A. without phosphates	120	28	31
,, ,,	C.A. without nitrogen	120	19	27
,, ,,	Double C.A.	120	14	31
,, ,,	C.A.	120	13	31

C.A. = complete artificials.

"STREAK" DISEASE OF THE BROAD BEAN AND CULINARY PEA.

During the early summer of 1920, diseased broad beans and culinary peas were observed growing on an allotment near to a nursery. The soil consisted of scrapings from tomato houses mixed with old cucumber soil. The similarity of the lesions to those obtained in the preceding inoculation experiments with *Bacillus lathyri* suggested that the causal organism was the same. Isolations yielded a yellow bacillus identical with *B. lathyri* which readily attacked the bean and pea and produced "Stripe" lesions when pricked into young tomato plants.

Symptoms.

(1) *Broad bean.* Dark purple brown spots and patches appear on the leaves, petiole and stem. The leaf spots, while small at first, soon enlarge and spread over the lamina, which becomes distorted and withers. Spots and blotches on the stem spread rapidly and become long broad streaks. The pods are disfigured by spots, blotches and streaks and finally become completely smothered with lesions.

(2) *Culinary pea.* The base of the stem first shows typical lesions, which appear as small black sunken spots, blotches and streaks, but the infection soon passes up the stem covering leaves, petioles, tendrils, stem and pods with lesions. The tissues round the lesions gradually turn yellow and then brown. Finally the plants turn yellow, wither and die prematurely. The organism may enter the plant either by means of the root or through the stoma at the base of the stem, which it reaches in water splashed up from the soil. Within the plant the pith is browned and destroyed and in the hollow parts of the stem, the cavities are lined with dead cells.

SUMMARY.

The organism which produces "Stripe" disease of the tomato is shown to be the cause of a number of "Streak" diseases of other plants, namely sweet pea, culinary pea, broad bean, french bean, red clover, lucerne, lupin, vetch, sainfoin and potato.

REFERENCES.

(1) **Manns, T. F. and Taubenhaus, J. J.** (1915). *Gard. Chron.* LIII, 215.

(2) **Paine, S. G. and Bewley, W. F.** (1919). *Annals of Applied Biol.* VI, 183.

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THE HEALING OF WOUNDS IN POTATO TUBERS AND THEIR PROPAGATION BY CUT SETS

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(With 3 Text-figures.)

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INTRODUCTION.

THE methods by which cut surfaces of potato tubers heal over so that the tissues within are again protected from excessive evaporation and against the entry of pathogenic organisms, have been made the subject of a somewhat extended investigation. The general question as to the methods by which injuries in parenchymatous tissues are repaired, which has been discussed elsewhere (12), will probably receive more elucidation through the intensive study of a few special cases than from a more extended survey of a wider number. The potato tuber provides parenchymatous tissues in a form exceptionally suitable for such investigation, and it is hoped that the special study of this particular case will also have some bearing on the more general question of the resistance of plant tissues to wound injury. At the same time the restriction of the investigation to the potato tuber has enabled a certain amount of attention to be directed to other questions of considerable general interest. Data are given below as to the relative activity of the processes of wound reparation in different varieties of potato. These data may have a certain interest in relation to the behaviour of the varieties, both as regards their suitability for propagation by cut sets and their resistance to disease, and possibly even as to their general vigour of growth. It is too early to assess the value of these data in this connection but they can at least be placed upon record. Finally the close study made of the natural process of wound healing has thrown some light upon the practice of propagating potatoes by means of cut sets. Some little discussion of this practice is therefore included and an attempt made to define certain conditions which may militate against successful propagation by this method.

It will be necessary first to describe in some detail the method by which cut surfaces in the potato tuber are normally healed. Experimental investigation seems to throw considerable light upon the conditions essential for the operation of the mechanism, and these will be discussed before the question of propagation by sets is dealt with.

The view advanced in an earlier paper (12) will be further developed below, namely that in the process of healing of an exposed parenchymatous tissue, certain processes follow in regular order. First the walls of the cells at the surface of the exposed tissue become covered with a deposit of a fatty nature (suberin). The deposit forms relatively rapidly and as a result of its formation the intercellular spaces of the tissue below the exposed surface are again cut off from the external atmosphere. This process is therefore spoken of as a preliminary "blocking" of the cut surface. An aqueous sap containing solutes collects behind the blocked surface and the result is that the living cells behind the blocked surface commence to divide actively, forming the meristem which, because the new cork layer arises as the result of its activities, is known as the cork phellogen.

I. THE "BLOCKING" OF THE CUT SURFACE.

Upon cutting a potato tuber across and leaving the cut surface exposed to air certain changes in the tissue are indicated by the appearance of the cut surface. The exposed surface quickly darkens in colour, the change being due to oxidation products resulting from the action of oxidising enzymes; thus the colour change proceeds more rapidly if the cut surface is exposed to an anaesthetic such as chloroform, but fails to occur if the tissue is exposed to a jet of steam for a few minutes immediately after cutting. Later this superficial black or brown stain is covered by a white, partially crystalline deposit, consisting of inorganic salts, starch, etc. left as the sap upon the cut surface evaporates. If the cut potato is exposed to sunlight or to very dry air, this outer crust of dried matter becomes exceedingly hard and later cracks violently, the cracks opening up avenues into the uninjured tissue behind.

When sections made at right angles to the cut surface are examined under the microscope, another result of the exposure of the surface is revealed. Within a period of some 12 to 36 hours at room temperature the walls of the cells in the close neighbourhood of the cut surface, though not necessarily the outermost cells of all, appear darker owing to a brown deposit upon them. The brown deposit is different from that found within the surface cells as the result of the enzyme action previously mentioned, and is noted only upon the walls. Appel(1) was the first to realise the significance of this layer, which he pointed out blocked the healthy tissues within from the penetration of fungi or bacteria. The time that this deposit takes to form is of considerable importance as, until it is complete, potentially pathogenic organisms may find an entrance to the healthy tissue within.

The Nature of the Blocking Deposit.

This deposit under most conditions (certain exceptional cases will be discussed later (p. 105)), forms a continuous layer along every wall of every cell at a certain depth below the injured surface. Sometimes it extends only over a layer one or two cells deep, in other cases it extends to a depth of seven or eight cells. In either case it is a continuous barrier between the protoplasts, cell walls and intercellular spaces of the healthy tissue within, and the atmosphere outside. It is therefore of the utmost importance to know its nature and the conditions of its formation.

The problem has been discussed previously(12) and the conclusion reached that it is a deposit of fatty nature, probably akin to suberin (Priestley(10)). In view of the importance of the question the matter will be rediscussed here in the light of further evidence. The staining reactions of the deposit certainly suggest the presence of fats. Thus it frequently stains well with Sudan III (used throughout the work in a 0·2 per cent. solution in alcohol mixed with an equal volume of glycerine; the sections are warmed in this reagent upon a microscopic slide until the alcohol commences to boil), but it is usually a browner red with the reagent than the typical red stain given by the suberin lamellae of the true cork cells that are formed later below this deposit. With osmic acid the deposit blackens slowly so that the presence of unsaturated fatty acids may be suspected. The Sudan III reaction is best given by a glyceride or neutral fat; while free fatty acids seem to stain very ineffectively with this reagent when it is used for the microchemical examination of tissue. The presence of glyceride and of unsaturated fatty acids is therefore indicated by these reactions. Very interesting further evidence is supplied by the behaviour of sections stained in

Grubler's Nile blue sulphate (1 per cent. aqueous solution) followed by washing in *very dilute* alkali or in aqueous sodium carbonate (1·5 per cent.). The suberin deposit then shows two regions, an outer region which is pink or mauve in colour, and an inner region running right across the deposit where the stain remains deep blue.

As the salts of Nile blue are deep blue in colour whilst the free base is red, this reaction certainly suggests that the inner layers of the deposit contain considerably more free acid than the outer portion. Staining experiments with indicators have also suggested that the inner portion of this deposit is more acid in reaction than the tissue within, thus the deposit stains deep red with methyl red (P_H 4·4–6), whilst the cells within give a yellow reaction.

The deposit also stains very deeply with many stains that have been successfully used for suberin, notably gentian violet and safranin, but these staining reactions though useful in investigating the distribution of the deposit cannot supply much evidence as to its nature. The deposit is soluble in hot alkalis, disappearing fairly easily on boiling in 5 per cent. aqueous potash and still more easily in alcoholic potash. After its removal the cellulose walls in this region give the blue reaction with chloriodide of zinc (used according to Artschwager's method (2)) but so long as the deposit is present the walls covered by it fail to give the cellulose reactions and resist hydrolysis by concentrated sulphuric acid. These reactions are clear evidence for the presence of some fatty substances in the deposit; doubtless other pigmented substances are present with them, derived probably from oxidation proceeding in the injured or dying cells. Additional evidence for the presence of fatty substances is also supplied by the following experiments.

It was thought that if fatty acids collecting at the cut surface were responsible for the deposit, then some evidence of the presence of these fatty acids would be obtained by placing the cut surface at once in contact with aqueous alkali. On May 22nd ten potatoes were halved, one set of halves thus obtained was cut into thin slices and these were covered with an aqueous solution of potassium hydrogen carbonate in a shallow dish. The other set was similarly sliced and immersed in a solution of calcium hydrogen carbonate. After half an hour the liquid was poured off from each set and fresh solutions of the same kind added and allowed to cover the slices for 36 hours.

The potassium solutions collected were fairly clear, they were filtered, the filtrate acidified and in each case extracted with pure ether (redistilled from stick potash). On evaporation of the ether a very small quantity of fatty acid was left, 0·008 gm. from the two filtrates together, slightly more being obtained from the first extract than from the second.

The calcium solutions contained a distinct precipitate and after filtration both precipitate and filtrate were acidified and the acid solution extracted with pure ether. In this case 0·01 gm. of fatty acid was obtained from the acidified precipitate and a negligible quantity (0·0001 gm.) from the acidified filtrate. As in the other case the greater yield of fatty acid was obtained from the liquid poured off at the end of the first half hour.

These experiments show that at the exposed surface of cut potatoes, fatty acids forming water-soluble potassium salts and insoluble calcium soaps collect within the first half hour after cutting. The quantities are very small, and apparently under the condition of the experiment new supplies do not continue to diffuse to the cut surface. These results are in agreement with the very extensive data now provided by Hansteen Cranner's investigations (4, 5, 6), which seem to place beyond doubt the presence of fatty substances in the wall and surface layers of the protoplasts of typical parenchymatous tissues. The experiments that are now to be discussed show that the conditions under which this deposit forms at the cut surface are such as would favour the oxidation and condensation of fatty acids to suberin-like products.

The Conditions of Formation of the Deposit.

A review of the earlier experiments dealing with the subject (12) showed that the evidence was strong for concluding that this deposit would be formed only in the presence of air. This conclusion was supported by our own experiments with cut surfaces left under boiled water, or under some substance preventing access of air, such as paraffin wax, when the deposit did not form.

On March 23rd potatoes were cut across under water and the cut pieces immediately placed in an atmosphere of hydrogen. They were left in this atmosphere until April 2nd, other pieces of the same potatoes being under another bell-jar in air. On April 2nd, when the pieces in the air had the normal deposit over two layers of cells and the beginnings of a cork phellogen visible beneath, the pieces in hydrogen were also examined. Microchemical reactions for fats were obtained from the contents of the cells near the cut surface but the walls of the cells appeared to be free from the usual brown deposit and with very few exceptions gave no reaction for fat. These pieces were then taken out of the hydrogen and left in moist air until April 5th when the fat deposit could be found on the walls of one to two layers of cells, and a cork phellogen, absent on April 2nd, was also appearing in various places beneath this layer.

This deposit upon the walls of some layers of cells beneath the cut surface exposed in air has now been seen on very many occasions. It invariably precedes the appearance of the cork phellogen, it forms in air whether the cut surface is in the light or in darkness, and it forms in damp or dry atmospheres though in sunlight or a very dry atmosphere it is irregular in distribution for reasons that are discussed later. Its rate of formation varies with the variety of potato, with temperature and probably with other conditions, but all attempts to obtain a quantitative expression of its rate of formation have so far failed, as the appearance and formation of the deposit depend upon microchemical observation that cannot be made sufficiently precise. This is unfortunate as undoubtedly it is a point of considerable practical importance to know after what time in a given variety under given conditions this layer may be dense enough to prevent the entrance of pathogenic organisms. Observation suggests however, that the rate of formation of this layer in a variety is governed by the same factors that later determine the rate of formation of the cork meristem. As the latter rate admits of rough quantitative expression, the data given later for cork formation probably indicate the relative speed at which the same varieties produce the first barrier between injured tissue and external environment.

If the observations and experiments described above and previously summarised are considered as a whole they provide a considerable body of evidence for regarding this initial barrier as a deposit of fatty substances, containing some neutral fat but in the main free fatty acids; these substances accumulate on the cell walls as they oxidise and condense into larger molecular aggregates relatively insoluble in fatty solvents and removed only by boiling in aqueous or alcoholic potash. These oxidation and condensation products form a solid film which is normally continuous and unbroken at a certain depth within the injured surface. This solid film has many of the properties of a suberin lamella, properties that have been considered elsewhere (Priestley and North (11)), and will not only prove an effective barrier to the entry of most pathogenic organisms but will also restrict materially the loss of water by evaporation. Gaseous exchange such as is essential to the maintenance of aerobic respiration will also be impeded by this layer.

II. THE CORK MERISTEM.

Within a few days of the formation of the suberin or fatty deposit described in the previous section, the cells just within the surface thus blocked show further changes. In the first place they rapidly lose their starch content, and judging by staining reactions a certain amount of starch is being converted into fatty acid. There then arises within this region by divisions running roughly parallel to the blocked surface, a layer of actively dividing cells. The outer cells of this meristem soon lose their original dense protoplasmic contents which evidently undergo rapid chemical change. Substances partly fatty in nature are produced and these are deposited all round the wall of each individual cell as a characteristic suberin lamella, while the original thin wall, of doubtful constitution but probably containing relatively little cellulose, is also impregnated with the deposit, which gives in the most definite manner all the microchemical reactions of suberin. Thus below the original surface blocked with a fatty, suberin-like deposit (Fig. 1 *a*), there is now a new and much more stable barrier established cutting off the intercellular spaces within from the external air. This new barrier consists of a continuous sheet of tabular-shaped cells, closely joined

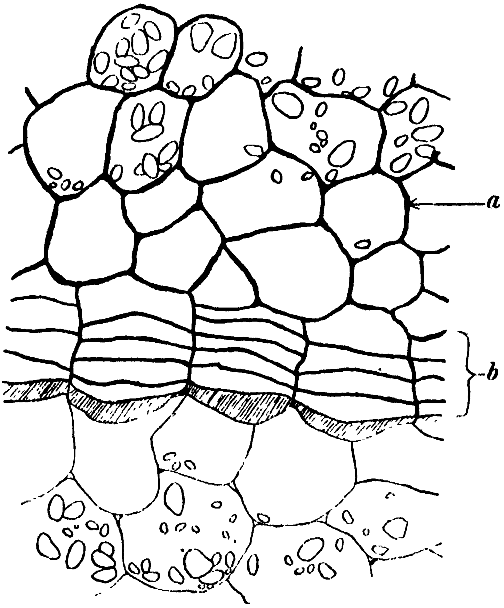

Fig. 1. Tinwald's Perfection. Section at right angles to cut surface 12 days after cutting, the cut surface uppermost.

together without intervening air spaces (Fig. 1 *b*). Beneath the layer of cork cells so formed, the inner layer of the original meristem remains still active as the cork phellogen, so that for some time the number of layers of cork continues to increase.

The blocking of the intercellular spaces by the original fatty deposit is an essential antecedent to the formation of the cork phellogen, but the reasons for this causal relation still require considerable elucidation. Experiment shows clearly that if the blocking of the parenchymatous tissues be prevented, *e.g.* by immersing the cut surface under water or by exposure in an atmosphere of hydrogen, then the phellogen fails to appear. If on the other hand the cut surface is blocked artificially, although the natural oxidation processes causing the deposit of suberin may thus be prevented, nevertheless beneath the blocked surface a phellogen appears (Priestley and Woffenden (12)), and a considerable number of layers of unsuberised regular brick-shaped cells are produced.

The question now arises as to what consequences may follow from the original blocking of a cut parenchymatous surface. The first blocking suggests that a slight flow of sap is occurring throughout the walls and intercellular spaces in the region of the cut surface. Usually this sap bathes all walls and spaces, so that as it dries in the neighbourhood of the air, the fatty residue it leaves behind forms a continuous layer and an efficient blocking surface. If the sap continues to flow, it must now begin to accumulate behind the blocked surface. Such a flow of sap certainly seems to occur from the neighbouring vascular tissues. Appel (1) pointed out, and we confirm his observation, that the bundles in the neighbourhood of the cut stain more strongly with basic dyes, especially gentian violet, this special staining power of the vascular strand disappearing further away from the cut surface. The observation is obviously suggestive in connection with the fact that the cork meristem is active sooner in the immediate vicinity of the vascular bundles. This point is well illustrated in text-fig. 2 which shows the general position of suberin deposit (*a*) and cork meristem (*b*) below the exposed surface of a potato cut across transversely. It will be seen that both these layers approach nearest to the surface in the immediate neighbourhood of the vascular tissue (*c*).

That the cell divisions in the meristem depend upon such a sap supply is particularly emphasized by Haberlandt's experiments (7), in which the capacity of very small pieces of potato tissue to give rise to cell divisions was under examination. These experiments show clearly that some substance necessary for this purpose diffuses from the phloem of the vascular tissue, and that in a small piece of potato tissue, if phloem is absent, the concentration of this substance in the sap irrigating the isolated piece of parenchyma is inadequate to promote cell division. Haberlandt speaks of this substance as a hormone, which stimulates cells to cell division; its nature remains as yet completely unknown.

The position of the meristem in the cut potato suggests that this substance accumulates in the walls and intercellular spaces behind the blocked surface, diffusing slowly there from the vascular bundles. In the case of cork formation on growing herbaceous stems evidence has been given elsewhere (12) for attributing the transport of this substance to the flow of sap from the xylem of the vascular bundle. In the case of the cut potato tuber no evidence has been obtained that there is any active flow of sap along the xylem of the tuber's vascular supply. All attempts to get more active meristem formation in cut sprouted tubers, as the result of driving water under considerable pressure into the cut end of the sprout have given negative results. The evidence is quite compatible with the idea that the substance is being carried to the cut surface by slow diffusion from the phloem.

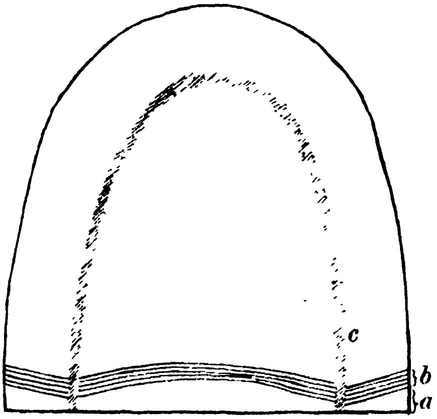

Fig. 2. Plan of cut potato—showing healing of cut surface.

At the same time the evidence is extraordinarily convincing that the original suberin deposit and the subsequent meristem are both formed as the result of something slowly diffusing *through the water* held in the walls and intercellular spaces. Thus if potato tubers are cut across in sunlight and left exposed in dry air for a few hours, instead of a continuous deposit of suberin, small brown patches are formed here and there at different depths below the cut surface. Appel (1), who first noticed this phenomenon, ascribed it to the lack of oxygen due to the difficulty in its diffusion through the drying surface, but the true interpretation is undoubtedly that the sap containing the fatty substances has never been able to form a continuous layer but has dried up rapidly leaving separated patches of fatty substance to be subsequently oxidised. This patchy layer of suberin is followed later by an irregular formation of meristem when the cut surface is in moister surroundings, because the new supply of sap as it oozes from the tissues and from the vascular bundles in particular, cannot form a continuous layer beneath a discontinuous blocked surface.

The supply of sap from the healthy tissues and notably from the vascular bundles of those tissues, is one factor in the production of the cork meristem but it is not the only one. Another factor of importance is undoubtedly the production of fatty acids below the blocked surface as the result of decomposition of the carbohydrate reserves under anaerobic conditions. The disappearance of starch is always associated with the appearance of free fatty acid beneath the blocked surface. The following experiment is somewhat instructive in this connection. Potatoes are placed in a bottle (*A* of text-fig. 3) connected by an oil manometer with another bottle *B*, the volume of air in *B* being adjustable by opening or closing a tap. The whole apparatus is placed in an incubator and adjusted until the manometer reading remains steady. By means of a Gillette razor blade inserted air tight through the rubber cork in *A* the potatoes are now cut repeatedly and the incubator closed and pressure

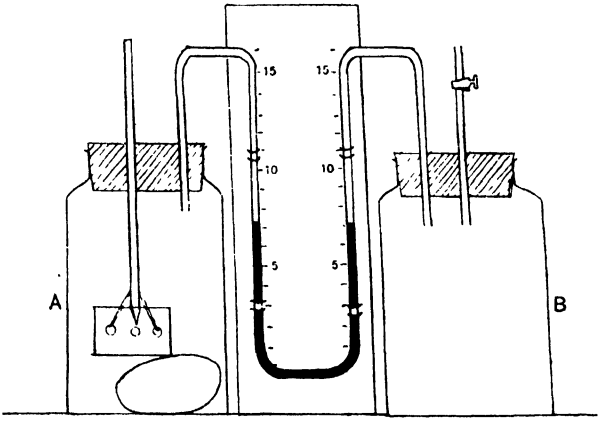

Fig. 3.

changes observed through the glass door, whilst the apparatus is maintained at constant temperature.

The changes in pressure that follow are always of the same type. First for a few hours the pressure in *A* falls, the oil rising in the left-hand arm of the manometer. This may possibly be associated with the intake of oxygen during the oxidation of the unsaturated linkages in the fatty substances in the exposed tissue. But after the first few hours the level of the oil falls in the left-hand tube and continues to do so for the next 24 hours; during this period more carbon dioxide is presumably being given out than oxygen absorbed. In the region below the cut surface during this period the rapid entry of oxygen is prevented as the fatty layer deposits, and below this layer the anaerobic decomposition of sugar with formation of fatty acid probably takes place.

Chemical considerations (Leathes(9) *loc. cit.* p. 107) suggest that the conversion of glucose into fatty acids can be accomplished without the addition of further oxygen, carbon dioxide being given off in the process and energy released, although the remaining carbon is now obtained as fatty acid in which the energy stored in calories per gram is relatively greater than in the original carbohydrate reserve.

The presence of these fatty acids below the blocked surface undoubtedly causes the local production of a medium bathing the adjoining protoplasts which is relatively more acid than the sap of the parenchyma itself. Observations upon different species of plants made by Mr Herklots in this department with the use of indicators show that this is a common phenomenon during cork formation and that when such a gradient of reaction (P_H) arises, a meristem frequently separates the tissues of different reaction. The same phenomenon is shown by the normal cambium of the vascular bundle arising between the phloem, a relatively alkaline tissue as Sachs observed long ago(13), and the relatively acid xylem (Atkins(3)). This raises a very general problem which it is hoped to deal with more adequately in a later communication from this laboratory.

Haberlandt(8) has invoked another factor in this connection which might replace the acid reacting sap below the blocked surface. He suggests that another hormone, or cell division stimulating substance is released from the wounded cells at the cut surface. But the cork meristem frequently appears for the first time at the end of seven or eight days, beneath a surface that has been blocked off from the injured cells at the surface for four or five days already, and in the subsequent weeks when cell division is active no such supply of "wound hormone" could reach the dividing phellogen. Furthermore, experiments in which the tubers have been cut across below a strong jet of water and half the cut surface held in a clamp under the jet, in one case for twenty minutes and in another for 2½ hours, have failed to demonstrate any retardation of cell division as a result of leaching away of the wound hormone. Thus on March 23rd potatoes were cut open in this manner and left under the jet of water for 2½ hours, the control half of the tuber being left in moist air in each case. On April 3rd both treated and control surfaces showed six layers of meristem (two suberised and four unsuberised in each case). Haberlandt also failed to obtain evidence that these wound hormones could be washed out from the cut cells of the potato. His positive results as the result of similar experiments with Kohlrabi seem better explained by the assumption that in this plant the washing of the surface with water interfered with its subsequent blocking, owing to the leaching away of the necessary fatty substances. (See Priestley and Woffenden (12).)

The activity of the phellogen produces a number of rows of very regular cells. The earliest formed rows to the outside are soon suberised and suberisation progresses slowly inwards, until as the rate of formation of the cells slows down the process of suberisation overtakes it and all cells except perhaps the innermost are seen to be suberised. It is a relatively simple matter to count the number of rows of cells thus present in the cork layer. Preliminary experiment showed that with uniform seed tubers of a given potato variety cut and placed under uniform conditions, cork formation proceeded at practically the same stage, as estimated by the number of layers of suberised and unsuberised cells formed. Some 24 tubers of the same variety were thus examined, sections being taken from the same region, the centre of the tuber in each case, so as to avoid the irregularity introduced by the neighbourhood of vascular tissue. The results obtained having varied very little, a comparison was now extended over a fairly representative series of varieties, the material being all Scotch seed obtained from Messrs Dobbie and Co., Edinburgh. The results are given in the accompanying table (Table I). The tubers in this experiment were left in the laboratory for 17 days under bell jars on moist filter paper. After the first few days, precautions were taken to protect them from direct sunlight, but some irregularities in these early results may be due to the irregular drying effect of occasional periods of exposure to the sun in the first two or three days.

Direct sunlight was avoided in the second series of experiments,

Table I.

Potatoes cut and left in laboratory 17 *days.*

Variety	Remarks	Numbers of layers of cells				
		Suberised meristem	Un-suberised meristem	Total meristem	Average of total meristem	Layers of previously suberised cells
Dargill Early	Variable meristem, not very regular. Begins 2nd row from surface	0–3	0–3	0–6	3	2
Rhoderick Dhu	Fairly regular. Varies in depth between 2nd and 4th row from surface	3–4	1	4–5	4	2–4
K. of K.	Fairly regular. Begins well below surface 2–3 cells deep	2–4	1–3	4–6	5	3
Crusader	Fairly regular. Varies in depth from surface	2–4	2–5	4–7	5	2
Arran Comrade	Irregular, occasionally no meristem. Occurs at varying depths 1st–3rd row. Meristem good when in 1st row	0–4	0–3	0–7	5	2–3
King Edward VII	Regular. In 2nd row of cells	2–4	2–4 (2–9 near veins)	3–11	5	2
America	Very variable. Practically no meristem in some but deep suberisation. Others regular meristem in 2nd or 3rd row	0–4	0–5	0–7	0 or 5	3
Kerr's Pink (W)	Variable. In one, regular meristem in 1st cell deep. Others irregular, at different depths, and 2–3 cells below surface	0–3	0–3	0–6	4	1–3
Kerr's Pink	Regular meristem, in 1st or 2nd row	2–4	1–4	3–6	5	1 (rarely 2)
Arran Chief (W)	Regular. Very near surface in 1st row	2–3	1–3	3–6	4	1
Arran Chief	Fairly regular in 1st or 2nd row	1–3	1–4	1–6	4	1–2
Bishop (W)	Fairly regular 2nd row	2–4	1–2	3–5	5	1–2
Bishop	Irregular. Occasionally no meristem, occurs generally 3rd row	0–3	0	0–2	3	3
Tinwald's Perfection	Fairly regular. Generally 2nd row deep	2–4	1–3	3–6	5	2
Immune Ashleaf	Fairly regular. Generally 2nd or 3rd row	0–2	0–3	0–5	4	2–3
Champion (W)	Regular, usually 2nd row deep	2–4	2–6	3–8	5	1–2
Great Scot	Irregular meristem. Occasionally none. Sometimes near surface, sometimes 3rd row	0–3	0–7	0–9	4	1–3
Resistant Snowdrop	Fairly regular. At different depths 1st or 2nd, occasionally 3rd row	2–4	1–4	3–7	5	1–2 (rarely 3)
Arran Rose	Regular, but of varying thickness, 2nd row	2–6	1–3	3–8	5–6	2
Majestic	Irregular or regular. Always near surface	2–3 (very rarely 0)	1 (–4 near veins)	3–6 (0)	3	1–2

(W) refers to seed tubers, grown from Scotch seed during 1921 at the experimental ground at Weetwood, Leeds.

Table II.

March 15–27th in greenhouse.

Variety	Remarks	Suberised meristem	Un-suberised	Average amount meristem	Depth of previous suberisation
Dargill Early	Fairly regular meristem	3–4	2–3	5	3–4
Roderick Dhu	Regular meristem	3–5	1–3	5	3
Crusader	Very little meristem—generally 0	0–1	0–3	2 (0)	4
K. of K.	Very regular meristem	3–5	0–1	5	2–3
Arran Comrade	Fairly regular	2–3	2	4–5	4
King Edward VII	Very poor and irregular	0–2	0–1	0–3	2
America	Meristem, where present is regular and usually quite near surface. In some sections have deeper suberisation (3) and little or no meristem	3 (occas. 0)	3 (occas. 0)	5	1–3
Kerr's Pink (W)	Regular (with few exceptions)	2–4	1–3	5	2–3
„ „	Not much difference from above	3	2	5	1–3
Arran Chief (W)	Generally no meristem	0–3	0–1	0 or 2	2–3
„ „	Fairly regular	1–4	1–2	1 or 4	3–4
Bishop (W)	Very variable, most have no meristem	0–4	0–1	4–5 or 0	2–3
„	Practically no meristem	0–2	0–1	0–2	3
Tinwald's Perfection	Regular	2–5	2–3	5	2
Immune Ashleaf	Not very regular. Occasionally absent	0–4	0–4	0 or 3	2–3
Champion (W)	Regular	3–4	0–1	4	3
Great Scot	Irregular, occasionally 0	0–4	0–1	3–4	3
Resistant Snowdrop	Regular	3–5	1–2	5	3
Arran Rose	Fairly regular	2–5	2–3	5	2–3
Majestic	Irregular	0–3	0–2	0 or 2	2

Table III.

Fifteen days, March 22nd–April 6th in greenhouse.

	Sprouted			Unsprouted		
	Sub-erised	Unsub-erised	Average total	Sub-erised	Unsub-erised	Average total
Dargill Early	0–4	0–1	0–2	0–3	0–1	0–2
Roderick Dhu	0–3	0	0–2	0–1	0	0
Crusader	2–4	0–1	3–4	2–4	0–1	3
K. of K.	0–2	0–1	0 or 2	0–2	0–1	0–1
Arran Comrade	0–5	0–2	2–3	0–5	0–1	2–3
King Edward VII	2–4	0–3	4–5	2–4	0–2	4
America	3–6	2	6	2–4	2–3	5–6
Kerr's Pink	2–4	0–2	4	2–4	0–2	4
Arran Chief	2–5	0–1	4	1–5	0–2	4
Bishop	0–3	0	0	0–3	0	0
Tinwald's Perfection	2–5	1–3	5	2–4	0–2	4
Immune Ashleaf	1–4	0–2	3–4	1–4	0–2	3–4
Great Scot	2–5	0–2	5–6	2–5	0–2	5
Resistant Snowdrop	1–4	0–1	3–4	1–3	0–2	3–4
Arran Rose	2–6	1–2	4–5	1–4	0–1	3–4
Majestic	0–2	0–1	0 or 2	0–2	0–1	0 or 2

Table IV.

Twelve days at 25° C. in incubator.

	Suberised	Unsuberised	Average total	Previously suberised
Dargill Early	2–4	1–3	5	2
Rhoderick Dhu	3–6	0–1	6	1–2
Crusader	0–3	0–1	2	3
K. of K.	0–1	0	0	2
Arran Comrade	0–4	0–2	0 or 3	2–3
King Edward VII	2–4	0–2	3–4	1–2
America	2–4	1–3	5–6	2
Kerr's Pink	1–6	0–3	4	2
Arran Chief	0–2	0–2	0 or 3	1
Bishop	1–2	1–3	2–3	2
Tinwald's Perfection	2–6	0–2	5–6	3
Immune Ashleaf	0–3	0–2	0 or 3	1–3
Great Scot	3–5	0–1	5–6	1–3
Resistant Snowdrop	2–6	0–1	4–5	2
Arran Rose	1–4	0–2	4	2
Majestic	1–3	1–2	3	1–2

Table V.

		Twelve days at 25° C.			Twelve days at 15° C.		
Variety	Remarks	Suberised meristem	Un-suberised meristem	Average amount meristem	Suberised meristem	Un-suberised meristem	Average amount meristem
Dargill Early	Fairly regular	2–5	0–2	4	0–1 occas.	1–3	2
Rhoderick Dhu	Regular	0–3	1–2	3	0	0–3	0–2
Crusader	— —	1–3	2	4	0–1 rare	0–3	0 or 2–3
K. of K.	At 25° C. 2–4 rows of cells at surface were unsuberised and below these were 2 rows suberised cells. At 15° 1–2 unsuberised, 2 suberised	0–1 occas.	0	0	0	0	0
Arran Comrade	Very variable amounts of meristem	0–3	0–2	0 or 3–4	0	0–3	0 or 2
King Edward VII	Fairly regular but occasionally none	0–3	0–2	3 occas. 0	0	0–2	0 or 2
America	Very good and regular	2–3	2–3	5–6	0	2–3	2
Kerr's Pink	Compressed appearance of meristem	0–3	0–2	4 occas. 0	0	0–2	0 or 2
Arran Chief	Near surface, irregular	0–3	0–2	0 or 3 rare	0	0–2	1–2
Bishop	— —	0–2	0–2	0–3	0	0 or 2	0
Tinwald's Perfection	Regular	2–6	1–3	5–6	0–1	0–3	2
Immune Ashleaf	Very variable. Regular in places. None or irregular in others	0–4	0–2	0 or 3	0	0 occas. 2	0
Great Scot	Fairly regular	2–5	0–3	5	0	2–4	3
Majestic	— —	0–2	0–1	0 or 2	0–1 rare	0–2	0 or 2

summarised in Table II, which were carried out in a similar manner, the cut tubers being left from March 15th until March 27th under bell jars in the greenhouse. The most striking feature emerging from these two tables, and seen also in the later tables, seems to be the poor production of cork meristem shown by the varieties Majestic and King Edward VII. Bishop, though irregular, is also very unsatisfactory, though it is interesting to note that tubers grown from Scotch seed for one year at Weetwood, Leeds, are producing better cork than seed tubers fresh from Scotland. So far as we can gather these three varieties, especially Majestic, would all be picked out by practical growers as relatively unsuitable for propagation by sets because they are "bleeders," *i.e.* found by experience not to heal well after cutting.

Potatoes are frequently sprouted before cutting, but to judge from the data supplied in Table III the previous sprouting has little effect upon the rate of healing of the tuber. These tubers were left from March 22nd to April 6th in the greenhouse and comparison is made between the half of the tuber with the sprout and the half without. The experiments so far described have been carried out at room temperature, without special precautions to keep it constant or to record it. Their results are in accordance with the experimental data supplied in Table IV, for cut potatoes kept for 12 days in an incubator at a constant temperature of 25° C. Finally, Table V shows the effect of temperature on the rate of formation of the meristem. It is interesting to see that the rate of formation appears to be approximately double when the temperature rises from 15° C. to 25° C. This temperature effect corresponds with the change of rate of a chemical reaction with temperature, and suggests that a chemical reaction may be the governing factor determining the rate at which the complex process of cork formation is proceeding.

Throughout these comparisons of cork formation in different varieties every precaution was taken to have conditions as constant as possible and tubers as comparable. In the case of three varieties (Kerr's Pink, Arran Comrade, and Tinwald's Perfection) experiments were carried out to see whether in relatively large and small potatoes appreciable differences in rate of cork formation were found. Details are not given but the result showed that no appreciable difference is to be found between large and small tubers when cut just before planting in the spring. On the contrary, some observations by Mr Cheveley in this department show that if the tubers be tested immediately after lifting in the summer then the small tubers are more active in cork formation.

In all these experiments the tubers have been cut across transversely. Some experiments were carried out to compare the behaviour of larger or smaller pieces of tuber, but the practical result appears to be that pieces such as are likely to be met with in practice, if cut from the same tuber have practically the same power of healing. When very small pieces are cut their behaviour is very different and depends upon the presence or absence of the phloem of the vascular bundle. The question has been fully explored experimentally by Haberlandt (7).

III. THE PRACTICE OF CUTTING POTATO SETS.

The data given in the previous sections may obviously throw some light on the best conditions under which potatoes are propagated from cut sets. The advisability of this practice is not under discussion. For various reasons it is still frequently employed and it is important to be able to define the most suitable practical conditions under which to carry it out.

When the cut tuber is placed in the ground, the most essential point is that it should be resistant to the entry of pathogenic organisms. A freshly cut surface is undoubtedly not resistant to organisms that penetrate the tissue rapidly; after the first layer of suberin has been deposited the surface is probably well protected provided the layer is continuous. To judge from the experiments described, so long as air has access to the cut surface this deposit of suberin will form, and provided the atmosphere is not too dry it will be formed in a continuous sheet. It appears then that tubers should be cut, left for a day or two in any moist warm place, preferably not piled in heaps but loosely spread out, and then planted; but unless the soil is very heavily infected with some pathogenic organisms the cut tubers would probably heal when placed directly in the soil, with very few casualties owing to disease reaching the healthy tissue before it is barred out by suberisation.

The Undesirability of Cutting in Sunlight.

Experiments have indicated clearly that if the potato tuber is cut and left for a time in sunlight, the continuous deposit of suberin is lacking and the potato therefore remains much more susceptible to disease. In cases such as growers report, where fields planted with potato sets of a variety like Majestic, have proved a complete failure, it would probably be well worth while to see whether this has been the cause of failure. During our own numerous experiments, notes have been kept of the occasions where cut tubers have been lost owing to fungus attack. In every case without exception, cutting in sunlight or exposure to too dry an atmosphere can be traced as the most probable cause. The dry atmosphere is particularly dangerous when the general temperature is low, and the suberin layer in consequence relatively slow in forming.

Some practical trials on this point are in progress, and the following results of a small scale trial at Doncaster by Mr G. C. Johnson, Instructor in Practical Horticulture, University of Leeds, are at least suggestive.

Variety: Great Scot. (1) Sets cut and exposed to bright sun, planted after 24 hours.
Yield 9 tons per acre.
(2) Sets cut and left in warm, damp, shady place for 24 hours.
Yield 17 tons per acre.
(3) Sets cut and planted immediately.
Yield 13 tons per acre.
(4) Control: whole sets seed size, planted.
Yield 18 tons per acre.

Apart from the need for the avoidance of direct sunlight or too dry an atmosphere for the cut sets prior to planting, and the advantage of an interval after cutting, before the sets are planted, wide difference in practical treatment would seem permissible.

The Practice of Liming Cut Sets.

It is a frequent practice to place the sets in powdered lime immediately after cutting, and considerable attention has been given to this question to see if the somewhat troublesome operation is justified by its results. In the first place we have been unable to ascertain definitely whether slaked lime or caustic lime should be used in this operation, potato growers having been found who are equally emphatic in favour of each of these alternatives respectively. We have therefore carried out experiments with both slaked and caustic lime, more frequently with caustic lime as the balance of evidence seemed to be in favour of this treatment. It might be anticipated that caustic lime would exert a considerable drying effect, which, if it did not prevent a continuous deposit of the fat might be of some value as favouring the change from the fat to the suberin, a change which undoubtedly is associated with loss of water from the fat (Priestley (10)). In fact, however, we have not been able to find that the caustic lime has any more effect upon the deposit of the suberin than the slaked lime, and neither form of lime seems to aid the formation of the normal suberin deposit. On the contrary the suberin layer is micro-chemically much more difficult to detect in the limed potatoes probably because, as might be anticipated from the experiment on p. 99, the fatty acid instead of condensing to suberin is to a considerable extent now precipitated at the surface of the injury as a calcium soap.

Observations made by growers had suggested to us that the limed sets lost less water in the air than the unlimed. These observations seemed to indicate that a better deposit of suberin had formed as a result of liming, but as the result of experiment we are driven to the opposite conclusion, viz. that limed sets lose more water than unlimed and that this is due to the deposition of the fatty acid in part as a calcium soap and the relatively weak blocking layer of suberin then developed. Experiment upon the loss of water from limed and unlimed sets prove difficult to carry out in a way free from objections. Experience showed that comparison should be made between pieces from the same tuber, left to dry under strictly comparable conditions, apart from the presence or absence of lime. The pieces needed to be of the same thickness and the loss of weight resulting from evaporation had to be calculated upon the area of cut surface exposed. There would be a certain loss of moisture from the normal uncut surface and in many experiments these surfaces were sealed by dipping in a paraffin wax of low melting point, in others cubes of potato tissue were used on which none of the original surface layer remained.

The final conclusion of a large amount of experiment upon this subject is that, under the conditions existing in practice, the procedure of liming, either with slaked or unslaked lime, has no practical value and that provided tubers are not cut in sunlight or left in too dry an atmosphere, a day or two's delay before planting is a more effective method of protection against disease. Similarly Shapovalov and Edson(14) conclude that the American practice of dusting with sulphur is unnecessary and drying of the tissues the thing to be avoided.

These practical conclusions are at present the subject of further experimental trial at the Manor Farm, Garforth, and at Doncaster, through the cooperation of the staff of the Agricultural Department of the University of Leeds. In view of the simplicity of the practical procedure that is suggested as most advisable for cutting sets, it is hoped that other agricultural experiment centres will compare the value of cut sets left under moist or dry conditions before planting. If our conclusions are supported by such experiment a hitherto unsuspected source of serious loss in present day practice with cut sets may be eliminated in future.

SUMMARY.

(1) The sequence of events during the healing of the cut surface of a potato tuber is carefully traced.

Apart from unessential colour changes, the first process in the healing of the tuber is the deposit of a fatty "suberin" layer, formed by the oxidation and condensation of fatty substances depositing from the sap drying up in the tissues at the cut surface.

(2) The condition essential to the formation of this layer is access of oxygen.

(3) If the cut surface is exposed in a moist atmosphere the layer is continuous; in a dry atmosphere, especially in sunlight, the layer may not be continuous.

(4) This layer forms within 24 to 48 hours. A few days later cork is formed below this suberin deposit as the result of cell divisions in an actively dividing layer, the cork phellogen.

(5) The essential factors promoting the activity of this phellogen appear to be the accumulation behind the blocked surface of sap containing substances diffusing from the vascular bundles, and the production of an acid reaction just below the blocked surface by the anaerobic conversion of sugars into fatty acids.

(6) The activity of the cork phellogen may be roughly estimated by the number of layers of cork produced. Comparative data are presented for a number of varieties of potato which show that Majestic, King Edward VII and Bishop are particularly deficient in this important activity connected with the healing of wounds.

(7) The bearing of these facts as to the process of wound healing is considered in relation to the practice of propagation by potato sets and two practical conclusions drawn. (1) Potato tubers should not be cut in sunlight or left in too dry a place. (2) The practice of liming potato sets appears to be without justification so far as its use in promoting the healing of the wound is concerned.

REFERENCES.

(1) APPEL, OTTO (1906). *Ber. der deutsch. Bot. Ges.* XXIV, 118–122.
(2) ARTSCHWAGER, ERNEST (1921). *Bot. Gaz.* LXXI, 400.
(3) ATKINS, W. R. GELSTON (1922). *Scient. Proc. Roy. Dubl. Soc.* XVI, 414–426.
(4) CRANNER, B. HANSTEEN (1914). *Jahrb. für Wissensch. Bot.* LIII, 536–598.
(5) —— (1919). *Ber. der deutsch. Bot. Ges.* XXXVII, 380–391.
(6) —— (1922). *Meldinger fra Norges Landbrukshiskole*, II, 1–160.
(7) HABERLANDT, G. (1913). *Akad. der Wiss. Berlin*, 318–345.
(8) —— (1921). *Sitzungsber. d. K. Preuss. Akad. der Wiss. Berlin*, I, 221–234.
(9) LEATHES, J. B. (1910). *The Fats.* London.
(10) PRIESTLEY, J. H. (1921). *New Phytologist*, XX, 17–29.
(11) PRIESTLEY, J. H. and NORTH, EDITH E. (1922). *New Phytologist*, XXI, 113–139.
(12) PRIESTLEY, J. H. and WOFFENDEN, LETTICE M. (1922). *New Phytologist*, XXI, 252–268.
(13) SACHS, J. (1892). *Bot. Zeit.* 1862 and *Ges. Anhand. über Pflanzenphysiologie*, I, 660–674, Leipzig.
(14) SHAPOVALOV, M. and EDSON, H. A. (1919). *Phytopathology*, IX, 483–496.

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EXPERIMENTS ON THE FEEDING OF EMBRYONATED EGGS OF *ASCARIS MEGALOCEPHALA* TO DOMESTICATED ANIMALS

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INTRODUCTION.

RESEARCHES carried out during the last few years on the life-history of certain species of Ascaris by a number of workers, Stewart (10), Ransom and Foster (8), Ransom and Cram (7), Yoshida (11), Fülleborn (1, 2, 3), Schwartz (9), Ortlepp (6), have shown that when embryonated eggs are fed to the normal host or to certain other laboratory animals, abnormal hosts, the contained embryos are set free when the eggs reach the intestine, and the larvae then migrate through the intestinal wall into the lymph vessels or the blood stream. In the latter they get carried to the liver and thence through the heart to the lungs.

By their presence in the lungs they may give rise to definite and well-marked pneumonic symptoms to which the animal may succumb. This holds good for both the normal and the abnormal host. For example, it has been found that the larvae of *A. lumbricoides*, normal to the pig and man, can set up pneumonia not only in pigs but in rats, mice, guinea-pigs, rabbits and goats; whilst Ortlepp (6) has found that the larvae of two species of Ascarids, *Polydephis attenuata* and *Ophidascaris filaria* from the Python, a cold-blooded vertebrate, can cause fatal pneumonia in a warm-blooded vertebrate such as a mouse.

Besides the extreme theoretical interest of these results, they are of great practical importance in that they reveal the possibility of the infestation of domesticated animals by the larvae of parasites not normally occurring in them.

The dog, the pig, the ox and the horse are specific hosts for Ascaris, the larvae of which, in the case of the first three of the above named, produce grave pulmonary lesions in experimental animals.

The point of economic importance was to ascertain how far the larvae of these parasites could produce pneumonic symptoms in sheep, and this led us to take up, in the first instance, the study of the Ascaris of the horse, *A. megalocephala*, as no work on these lines had been prosecuted on this species. The horse is herbivorous and it seemed within the realms of possibility that other herbivorous animals such as lambs might run the risk of pulmonary trouble simply by feeding on pastures infected with the embryonated eggs of *A. megalocephala*.

The results recorded hereafter show that the larvae of this species of Ascaris can produce pulmonary lesions in laboratory animals and that consequently *A. megalocephala* comes into line with the other Ascaris species in which migration through the lungs is a normal occurrence.

The experiments on two lambs described in the present paper were carried out at the farm of the Rothamsted Experimental Station, Harpenden. On behalf of Professor R. T. Leiper and myself I desire to express our indebtedness to Sir John Russell, F.R.S., Director of the Station, and to Mr S. J. K. Eames, the Farm Manager, for kindly placing the necessary accommodation and working facilities at our disposal.

MATERIAL.

Specimens of female *A. megalocephala* were obtained from various sources, and after opening up the body cavity the two uteri were removed. As a rule the anterior third of each was taken and the remainder rejected as probably containing eggs incapable of completing their development. The procedure recommended by Ransom and Foster(8) for the culture of the eggs was followed. The portions of uterus were put into 2 per cent. formalin and cut into small pieces, and then lightly rubbed up in a mortar so as to force out the eggs and break up the larger egg masses. The resulting mixture was put into Petri dishes and incubated sometimes at 22° C. and sometimes at about 30° C. In nearly all cases there was a varying proportion of eggs, which failed to develop, but in most cases very large numbers of embryonated eggs were obtained.

INFECTION EXPERIMENTS.

Experiment 1. Two mice were fed with biscuit moistened with a suspension of many thousands of washed embryonated eggs. The animals had remained without food over night before being given their first feed of infective material. They were fed on two successive days and must have swallowed a very large number of embryonated eggs.

On the fourth day both of them showed signs of illness and were huddled up in the corner of their box.

On the fifth day both were found dead, with blood exuding from the nose and mouth, which had evidently been coughed up from the lungs. On opening up the body cavity the liver and lungs were both seen to be abnormal in appearance, the lungs showing many bright red spots, whilst the liver had numerous creamy spots scattered over its surface. Press preparations of portions of both liver and lungs from the region of the diseased spots showed numerous Ascaris larvae actively moving in the tissue. One mouse was fixed entire after being skinned, and was put aside for later examination. From the other, portions of the liver and lungs were taken and fixed. These were embedded in paraffin in the usual way and sections were made which showed the larvae in the tissues.

There could be no doubt that the mice had succumbed to pneumonia set up by the larvae of *A. megalocephala* in lungs, and this positive result showed conclusively that the passage of larvae through the lungs is normal for *A. megalocephala* as for *A. lumbricoides*, *Belascaris* and *Toxascaris* spp., *Polydephis attenuata*, *Ophidascaris filaria* and *A. vitulorum.*

EXPERIMENT ON SKIN PENETRATION.

The following note on an experiment to test the possibility of skin penetration by the larvae of *A. megalocephala* may quite well be given here. The experiment was prompted by the fact that Yoshida (11) claims to have infected a guinea-pig through the skin with larvae of *A. lumbricoides* obtained from the liver of another experimental guinea-pig, and placed on the shaved skin of the abdomen and in the region of the shoulder. Another Japanese worker, Asada, is cited by Fülleborn (2) as having succeeded in getting larvae of *A. lumbricoides* to penetrate the intact skin of mice, rats and guinea-pigs. Ortlepp (6) found that the larvae of the two species of Ascarid *Polydephis attenuata* and *Ophidascaris filaria* were incapable of penetrating intact skin when tested by the method revised by the present writer, Goodey (4, 5). Ortlepp, by the way, gives the wrong reference in his text to Fülleborn's paper; it should be Fülleborn (1921) not (1922).

In the experiment recorded here the larvae of *A. megalocephala* were obtained from the droppings of a mouse three and a half hours after it had been fed with a heavy dose of embryonated eggs. The droppings were teased up in a little distilled water and the larvae which swam about freely were picked up by means of a capillary pipette. In this way a fairly large number were collected in a small drop of water, which was transferred to the surface of a portion of skin removed from the abdomen of a very young rat, which had been stretched and pinned over a hole in a piece of cork, and then floated on the surface of N. saline kept at 37° C. in the manner described by the author(4, 5).

The *A. megalocephala* larvae were seen to show a certain amount of spasmodic movement, but there was no definitely downward movement such as was observed in the experiments with larvae of *Necator americanus* described in the paper referred to above. The drop of water containing the larvae was allowed to dry up gradually and all the conditions under which larvae of *Necator americanus* penetrated the skin were arranged, but at the end of the experiment none of the larvae had penetrated, and the majority of them were removed from the skin by flooding it with a drop of water and then sucking this up in a pipette. It would thus seem that the hatched larvae of *A. magalocephala* are incapable of penetrating intact skin.

EXPERIMENTS WITH LAMBS.

Experiment 1. A male lamb, four days old, was obtained and taken to Rothamsted Experimental Station Farm, where throughout the course of the experiment it was bottle fed.

On two successive days it was fed with a heavy suspension of embryonated eggs added to the milk in the feeding bottle; many thousands of eggs were given. The weather was very cold during the period of the experiment and the lamb, although well cared for and protected from draughts, seems to have contracted a chill, for it died seven days after the first feeding with eggs.

On opening up the animal at the post mortem, the only sign of disease was seen in the left lung which was very much congested and dark red in appearance. The right lung was quite healthy and so also was the liver.

Numerous press preparations were made of portions of both lungs and liver, and were examined microscopically, but no Ascaris larvae were found.

A careful search was also made through portions of the intestine but with negative results.

Experiment 2. About ten days after the death of the first lamb another one was obtained from the same source. This was a female ten days old, and as before, it was bottle fed during the course of the experiment.

A very thick culture of washed embryonated eggs was fed to this lamb by means of a teaspoon, the masses of eggs being spooned up from a saucer. The feeding was completed by washing down with a little distilled water from a bottle. This method of giving eggs was very successful. Fourteen days later the lamb was given a second lot of eggs by the same method, and two days after this a third feed was administered. In these three feeds the lamb received enormous numbers of embryonated eggs, the contents, in fact, of three culture dishes, each six inches in diameter, and each culture so rich in eggs that one could not see through it. It must have taken over a million eggs. The animal did not, however, at any time during the course of the experiment suffer the slightest inconvenience, and at no time did it exhibit any signs of pulmonary disturbance. Droppings were collected 24 hours after the first feed, and these were found to be extremely rich in eggs. A few free larvae were also found in the droppings, thus showing that a certain amount of hatching had taken place. Empty egg-shells and partially hatched eggs were also found.

A count was made to determine the numbers of (1) unhatched embryonated eggs, (2) partially hatched egg, and (3) empty shells. The result of the count showed that for every 100 unhatched embryonated eggs in the droppings there were about 30 partially hatched, and only five empty egg-shells.

A similar count was made on the eggs passed in the droppings collected 24 hours after the third feed. On this occasion also eggs were extremely plentiful in the faeces, and a few free larvae were also found. The proportions of unhatched embryonated, partially hatched and empty egg-shells were the same as in the previous count, *i.e.* unhatched 100, partially hatched 30, and empty shells 5.

The two counts show that comparatively few of the embryonated eggs administered had hatched out in the intestine. In view, however, of the enormous number of eggs administered in the three feedings it would not have been surprising had the lamb shown signs of pulmonary disturbance, even though only 5 per cent. of the embryonated eggs had hatched.

The results of these two experiments are interesting and lead one to the conclusion that lambs are not susceptible to pulmonary trouble due to the migrating larvae of *A. megalocephala.*

It is not claimed, however, that this is a wholly conclusive result inasmuch as two attempts to set up pulmonary symptoms in mice proved failures for some unknown reason, and in this connection it may be mentioned that some of the workers already cited have recorded negative results in some of their experiments. It is thus possible that the failure in the present instance may come in this category of experiments, though I do not think this a probable explanation.

It is interesting to note in this connection that Ransom and Foster (8), p. 42, in their one recorded experiment on a lamb which they fed with embryonated eggs of *A. suum*, do not mention the occurrence of any pneumonic symptoms, and one naturally presumes that no such symptoms manifested themselves, or they would have been noted as in the other cases where such symptoms arose.

CONCLUSIONS.

1. The larvae of *A. megalocephala* are capable of causing fatal pneumonia to mice in their migratory course through the lungs.

2. Attempts to cause pulmonary disturbance in lambs by feeding them with embryonated eggs of *A. megalocephala* proved unsuccessful and it would seem that lambs are not susceptible to such trouble. The need for further work on these lines is indicated.

REFERENCES.

(1) FÜLLEBORN, F. (1921). Über die Wanderung von Askaris und anderen Nematodenlarven in Körper und intrauterine Askarisinfektion. *Arch. f. Sch. u. Trop. Hyg.* Bd. XXV, 146.

(2) —— (1921). Askarisinfektion durch Verzehren eingekapselter Larven und über gelungene intrauterine Askarisinfektion. *Arch. f. Sch. u. Trop. Hyg.* Bd. XXV, 367–375.

(3) —— (1922). Über Infektionversuche mit Toxascaris. *Arch. f. Sch. u. Trop. Hyg.* Bd. XXVI, 59.

(4) GOODEY, T. (1922). A Simple Method of Experimentation for Skin Infection with Hookworm Larvae. *Proc. Roy. Soc. Med.* XXV, 19–20.

(5) —— (1922). Observations on the Ensheathed Larvae of some Parasitic Nematodes. *Ann. of Applied Biology,* IX, 33–48.

(6) ORTLEPP, R. J. (1922). On the Hatching and Migration in a Mammalian Host of Larvae of Ascarids normally Parasitic in Cold-blooded Vertebrates. *Journ. Trop. Med. and Hyg.* XXV, No. 8, April 15th, 97–100.

(7) RANSOM, B. H. and CRAM, E. B. (1921). The Course of Migration of Ascaris Larvae. *Amer. Journ. of Trop. Med.* I, No. 3, 129–159.

(8) RANSOM, B. H. and FOSTER, W. D. (1920). Observations on the Life-History of *Ascaris lumbricoides*. *Bull.* 817, U.S. Dept. Agric., Washington.

(9) SCHWARTZ, B. (1922). Observations on the Life-History of *Ascaris vitulorum*, a parasite of Bovines in the Philippine Islands. *Phil. Journ. of Science,* XX, No. 6.

(10) STEWART, F. H. (1917). On the Development of *Ascaris lumbricoides* Lin. and *Ascaris suilla* Duj. in the Rat and Mouse. *Parasitology,* IX, No. 2, 213–227.

(11) YOSHIDA, S. (1919). On the Migratory Course of Ascarid Larvae in the Body of the Host. *Journ. Parasitology,* VI, 19–27.

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SOME PRELIMINARY INVESTIGATIONS ON THE RELATIONSHIP OF PROTOZOA TO SOIL FERTILITY WITH SPECIAL REFERENCE TO NITROGEN FIXATION

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(With 1 Diagram.)

I.

ALTHOUGH the presence of protozoa in soil was observed by Müller (10) as far back as 1887, real advance in our knowledge concerning their activities and importance in relation to soil fertility dates back only to 1909, when Russell and Hutchinson (13) announced their "Protozoa" theory of the partial-sterilisation of soils. This theory has been a subject of much speculation and discussion as to the nature of limiting factor.

"PROTOZOA" THEORY.

Russell and Hutchinson (13) considered "that the soil population is complex and that some of its numbers act detrimentally on the bacteria which produce plant nutrients: these detrimental forms are more readily killed than the useful bacteria, with the result that the new population produces more ammonia and nitrate than the old one. The detrimental organisms are provisionally identified with protozoa."

The evidence put forth by them in support of their theory tended to show that protozoa do work as phagocytes on soil bacteria and the increased fertility of the partially-sterilised soil was due in part to the availability of a large amount of organic matter left behind in the form of dead bodies of organisms killed by partial sterilisation.

EFFECT OF PROTOZOA ON MASS NUMBER OF BACTERIA IN SOIL.

Russell and Hutchinson's experiments showed a depressing effect on the mass number of bacteria when normal soil containing protozoa was added to the partially-sterilised soil. Cunningham (1) and Goodey (4) also recorded observations of similar nature and confirmed Russell and Hutchinson's views. Waksman (15) too admitted that certain of the trophic fauna acts detrimentally on soil bacteria.

Martin and Lewin(9) established that protozoon occurs normally in a trophic state in soils.

Cutler's(2) ingenious method of determining active numbers of protozoa in soil affords direct evidence that they occur in an active and trophic form, and as such are able to feed upon bacteria, bringing about a depression of their numbers.

Cutler, Crump(3) and Sandon(3*a*), in their daily counts for 365 days, have succeeded in demonstrating this depressing effect of amoebae on bacterial numbers[1].

Greig-Smith(5), on the other hand, denied the functioning of protozoa as a limiting factor to soil bacteria in the soils he examined. He suggested instead that "Agricere"(6) forms a water-proofing layer around the soil particles, and is destroyed or segregated at the points of soil particles by partial sterilisation. These conditions, however, could not be reproduced experimentally, and the wax instead of forming a water-proofing layer served as an excellent food material for the bacteria.

Sherman(14) also joined hands with Greig-Smith in opposing the phagocytic action of protozoa on soil bacteria. His results show enormous decrease in bacterial numbers in presence of protozoa in a partially-sterilised soil, yet he concludes that protozoa have no action whatsoever on soil bacteria. The figures he obtained in the case of pure cultures were very contradictory. In one test the bacterial numbers increased when protozoa were introduced, while in another similar test they decreased. The incubation period was only eight days, hardly enough to give time to protozoa to establish themselves. Evidence is also lacking to show if the so-called "active" cultures were really alive and active after the inoculation from the artificial media to soil. It will be shown later that inoculation with protozoa is not a simple matter. The types of protozoa made use of were only ciliates and flagellates, missing the most important group the "amoebae," which are more destructive and are normally present in soils.

THE RELATIONSHIP OF PROTOZOA TO THE MOST IMPORTANT BACTERIOLOGICAL PROCESSES IN SOIL.

1. *Ammonification.*

In a partially-sterilised soil in which protozoa were absent Russell and Hutchinson(13) observed a great increase in ammonia production in comparison with the untreated soil. Cunningham's work also supported Russell and Hutchinson's observations. Hill (7), on the other hand, carried out a set of triplicate experiments and concluded that protozoa have no effect on ammonification. His results show 100 per cent. increase in ammonia production after 12 weeks in absence of protozoa, but this was more than offset by a difference in nitrate production. Again, however, it is not clear that the inoculation was properly carried out.

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[1] The inoculation experiments described by Cutler in this number of the Annals (*Ann. App. Biol.* 137) afford an interesting confirmation of this statement.

Lipman, Blair, Owen and McLean's (8) work on the possible influence of protozoa on ammonification contains large number of figures obtained by using dried blood, tankage, soluble-blood flour, cottonseed meal, soybean meal, wheat flour, corn meal etc., but does not clearly show whether the soil used for the experiment actually contained protozoa. From the text one finds no evidence of the existence of protozoa, either in the original soil, or the inocula used in the tests. It may be doubted also whether much reliance can be placed on results obtained with substances liable to great variations in their composition and not normally present in the soil.

2. *Nitrogen-fixation.*

The process of free nitrogen-fixation in soils is an important one, more especially in countries where on *Barani* (dependent on rain) soils of light sandy nature crops are taken year after year without any application of manure. After each crop the land is allowed to remain fallow for a considerable period—usually till the next sowing season—during which time the usual agricultural operations are carried out.

The effect of soil protozoa on this class of organisms has not been studied in detail. Hill (7) carried out an experiment in triplicate in liquid mannite solution as well as in heated soil to which protozoa had been added, and concluded that in liquid media protozoa feed upon azotobacter, checking their activities to a certain extent, while in the heated soil their presence is without any effect. He makes no reference to the fact that even if protozoa consumed azotobacter, it was simply a question of the nitrogen being transferred from the bodies of azotobacter to that of protozoa, since there is no evidence on record to show that protozoa are capable of bringing about denitrification. The utilisation of fluid medium involves so many factors that the results obtained by its use must not be too greatly relied upon: the depth of liquid, the tightness of the plug, the surface of the medium, the action of glass vessels and the question of aeration have all been found to have a tremendous effect on the results. The conditions are not at all comparable to those of soil.

II. EXPERIMENTAL PART.

The investigations recorded here deal with the studies of the influence of protozoa on azotobacter. The writer has made an effort to collect some preliminary data and determine the extent of the action of protozoa on this most important process of free nitrogen-fixation.

METHODS.

The protozoon cultures were isolated from different soils and grown with azotobacter and other bacteria on mannite agar of the following composition:

Mannite	20	grms.	Calcium sulphate	0·1	grms.
Calcium carbonate	5	,,	Mono-potassium phosphate	0·2	,,
Magnesium sulphate	0·2	,,	Agar agar	20	,,
Sodium chloride	0·2	,,	Water (distilled)	1000	c.c.

(Mono-potassium phosphate was separately dissolved in water and the reaction adjusted to 7·2 *p*H.)

The organisms isolated and made use of are as follows:

Table I.

Showing the detail of organisms used in the experiment.

No. of culture	Organisms present*
1.	Colpoda sp., Hartmanella hyalina, Flagellate sp., Azotobacter and other bacteria.
2.	Colpoda sp., Hartmanella hyalina, Azotobacter and other bacteria.
3.	Heteromita globosus, Azotobacter and other bacteria.
4.	Hartmanella hyalina, Sp. γ†, Azotobacter and other bacteria.
5.	Colpoda sp., Hartmanella hyalina, Ophidomonas sp., Azotobacter and other bacteria.
6.	Hartmanella hyalina, Sp. γ†, Azotobacter and other bacteria.
7.	Hartmanella hyalina, Flagellate sp., Azotobacter and other bacteria.
8.	Hartmanella hyalina, Flagellate sp., Azotobacter and other bacteria.
9.	Colpoda cucullus, Azotobacter and other bacteria.
10.	Azotobacter and other bacteria.
11.	Hartmanella hyalina, Azotobacter and other bacteria.
12.	Azotobacter (pure culture).
13.	Azotobacter (pure culture).
14.	Colpoda sp., Azotobacter and other bacteria.
15.	Colpoda sp., Hartmanella hyalina, Azotobacter and other bacteria.
16.	Colpoda sp., Azotobacter and other bacteria.
17.	Hartmanella hyalina, Flagellate sp., Azotobacter and other bacteria.
18.	Colpidium colpoda (with only one species of bacteria) and Azotobacter.

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* I am indebted to Mr H. Sandon of this laboratory for checking the identity of these cultures.

† New species described in the paper. See Reference (3 *a*).

Special care was taken to prepare culture No. 10, *i.e.* control. It contained all the species of bacteria present in the protozoa cultures. The separation of protozoa was carried out by heating a suspension of the mixed culture to 80° C. at which temperature all active forms of protozoa are killed, also by centrifugalising another similar portion and decanting of the upper liquid containing bacteria only. The two were then mixed and utilised as control.

25 c.c. of the media was placed in 250 c.c. Erlenmeyer flasks and after sterilisation allowed to cool in an inclined position so as to obtain slopes, 5 c.c. of sterile water was then added to each flask. (It is important to keep a good supply of moisture over the surface of the medium while the organisms are growing, otherwise the growth will not be uniform.)

Method of inoculation.

(i) In artificial media:

After the stock cultures had grown for about a week, and a thick scum was formed over the surface of the medium—at which time almost all the protozoa are in an encysted condition—three loops were taken for inoculation in each flask.

It is rather important to let the protozoa encyst before transferring them on to fresh medium since there is a large percentage that die if transferred in an active condition, and the inocula will not be uniform.

(ii) Sand cultures:

The method employed for inoculation was that suggested by Mr D. W. Cutler. He found that if protozoa are added to soil or sand in the ordinary way they usually do not grow; one has to be very careful in making inoculation in soil or sand cultures.

The method suggested and employed successfully on previous occasions was to rub in a portion of the scum in the requisite quantity of the fluid media so as to ensure the distribution of the organisms throughout the whole media, and then spray the liquid on to the surface of the sterile sand, so that it penetrated into the entire column of the sand.

Preparation of sand.

A quantity of sand was digested with strong hydrochloric acid for 24 hours and washed free of acid under the water tap and dried. Hundred gramme portions of this were then placed in 250 c.c. Erlenmeyer flasks and sterilised in an autoclave at 130° C. for 30 minutes. The moisture contents were made up by adding the liquid nutrient media used for inoculation.

Examination of cultures while the experiment was in progress.

Artificial culture medium:

Microscopic examination was carried out every fourth day to ensure that the protozoa were growing in the media.

Sand cultures:

A quantity of sand (0·5 grm.) was taken out at an interval of 7th and 14th day, and placed on a quantity of artificial medium in petri dishes with 1 c.c. sterile water, and examined after the growth had taken place.

Direct microscopic examination of a small quantity of sand with a drop of sterile water on the microscopic slide was also made and the protozoa were seen growing in an active condition.

In No. 10 (control) no protozoa developed.

Inoculations in sand were carried out with the help of Mr D. W. Cutler, who also examined cultures before inoculation as well as after the 7th and 15th day.

Incubation temperature and period.

The incubation temperature was the usual room temperature, viz. 20–22° C.

In the first series of experiments the incubation period was only ten days and on testing it was found that a portion of mannite was still present in the cultures, therefore in all the latter experiments the incubation period was 15 days.

Method of nitrogen estimation.

Kjeldahl's method was utilised in determining the nitrogen content of each culture. 20 c.c. of strong sulphuric acid was used for digestion, and after adding the excess of alkali the ammonia was distilled off, receiving the distillate in decinormal sulphuric acid. For titration decinormal sodium hydroxide was used. The indicator employed was tincture cochineal.

I am indebted to Mr H. J. Page, head of the chemical department, for giving me facilities to carry out the estimations in his laboratory.

Accuracy of the experiments.

Each experiment was carried out in duplicate, and in certain cases in triplicate. The results in most cases agreed to a single drop of decinormal sulphuric acid.

Series I. Artificial culture media.

(Incubation period 10 days.)

No. of culture	Organisms* present	Mgms. of nitrogen fixed per gram of mannite	Gain or loss	
			Actual	Per cent.
1	C.A.F.N.†	10·74	+0·14	+ 1·32
5	C.A.F.N.	11·03	+0·43	+ 4·06
2	C.A.N.	10·74	+0·14	+ 1·32
6	A.F.N.	10·88	+0·28	+ 2·64
7	A.F.N.	12·60	+2·00	+18·90
8	A.F.N.	9·74	−0·86	− 8·10
3	F.N.	10·74	+0·14	+ 1·32
11	A.N.	10·45	−0·15	− 1·42
9	C.N.	10·88	+0·28	+ 2·64
10	N. (control)	10·60	—	—

* For detail of organisms please see Table I.

† C. means ciliates; A. amoebae; F. flagellates; and N. nitrogen-fixing bacteria.

Series II. Artificial culture media.

(Incubation period 15 days.)

No. of culture	Organisms* present	Mgms. of nitrogen fixed per gram of mannite		Gain or loss	
		Parallels	Mean	Actual	Per cent.
1	C.A.F.N.†	11·89 12·32	12·1	+2·07	+20·1
5	C.A.F.N.	11·6 12·17	11·89	+1·86	+18·06
2	C.A.N.	11·46 12·03	11·75	+1·72	+16·7
15	C.A.N.	11·6	11·6	+1·57	+15·25
6	A.F.N.	11·17 11·03	11·1	+1·07	+10·39
4	A.F.N.	11·46 10·88	11·17	+1·14	+11·07
7	A.F.N.	12·6 12·89	12·75	+2·72	+26·41
8	A.F.N.	10·17 9·74	9·96	−0·07	− 0·69
17	A.F.N.	9·74	9·74	−0·29	− 2·81
3	F.N.	11·17 10·88	11·03	+1·0	+ 9·71
11	A.N.	10·74 10·88	10·81	+0·78	+ 7·57
9	C.N.	11·17 10·6	10·89	+0·86	+ 8·35
14	C.N.	12·89	12·89	+2·86	+27·77
10	N. (control)	10·31 9·74	10·03	—	—

* For detail of organisms please see Table I.

† C. means ciliates; A. amoebae; F. flagellates; and N. nitrogen-fixing bacteria.

Series III. Artificial culture media.

(Incubation period 15 days.)

No. of culture	Organisms* present	Mgms. of nitrogen fixed per gram of mannite		Gain or loss	
		Parallels	Mean	Actual	Per cent.
5	C.A.F.N.†	10·31 11·65 10·17	10·71	+1·12	+11·68
15	C.A.N.	11·03 10·60	10·82	+1·23	+12·83
7	A.F.N.	12·03 11·03 11·17	11·42	+1·83	+19·08
9	C.N.	10·6 11·11 10·6	10·77	+1·18	+12·3
14	C.N.	10·54 10·88	10·71	+1·12	+11·68
10	N (control)	9·59 9·59	9·59	—	—

* For detail of organisms please see Table 1.

† C. means ciliates; A. amoebae; F. flagellates; and N. nitrogen-fixing bacteria.

Series IV. Sand cultures.

(Incubation period 15 days.)

No. of culture	Organisms* present	Mgms. of nitrogen fixed per gram of mannite		Gain or loss	
		Parallels	Mean	Actual	Per cent.
1	C.A.F.N.†	4·58 4·58	4·58	-0·58	-11·24
15	C.A.N.	5·73 6·59	6·16	+1·0	+19·38
6	A.F.N.	5·44 5·16	5·3	+0·14	+ 2·71
17	A.F.N.	5·73 4·87	5·3	+0·14	+ 2·71
11	A.N.	6·59 6·3	6·46	+1·3	+25·19
14	C.N.	6·59 7·45	7·02	+1·86	+36·04
10	N. (control)	5·16 5·16	5·16	—	—

* For detail of organisms please see Table 1.

† C. means ciliates; A. amoebae; F. flagellates; and N. nitrogen-fixing bacteria.

A special experiment in triplicate was arranged to determine the extent of experimental error, the nitrogen estimations being carried out by three different workers. The results agreed very closely and the biggest difference in individual figures was 0·35 c.c. $N/10$ sulphuric acid, which means only 0·00050120 grm. of nitrogen in a set of duplicate or triplicate estimations. It may be pointed out here that this difference of 0·35 c.c. of *N*/10 sulphuric acid was experienced only in one case. In majority of tests it ranged between 0 and 0·2 c.c.

Mr R. L. Amoore, of the fermentation laboratory, Mr A. H. Bowden, of the chemical laboratory, and Mr A. N. Puri, of the soil physics department, kindly carried out the estimations for this special experiment, Series III, to whom my best thanks are due.

Series V and VI. Pure cultures of *Colpidium colpoda* and Azotobacter.

(Incubation period 15 days.)

No. of culture	Organisms* present	Mgms. of nitrogen fixed per gram of mannite		Gain or loss	
		Parallels	Mean	Actual	Per cent.
In artificial culture media:					
18	C.N.†	11·89 12·46	12·18	+ 1·22	+ 11·13
12	N. (control)	11·46 10·45	10·96	--	- -
18	C.N.	11·46 11·17 11·31	11·31	+0·91	+ 8·75
12	N. (control)	10·45 10·45 10·31	10·4	—	—
In sand cultures:					
18	C.N.	10·02 9·45 9·74	9·74	+2·68	+37·96
12	N. (control)	8·02 6·87 6·30	7·06	—	—

* For detail of organisms please see Table I.

† C. means pure culture of *Colpidium colpoda* (with only one species of bacteria) and N. means pure culture of Azotobacter.

SUMMARY OF RESULTS.

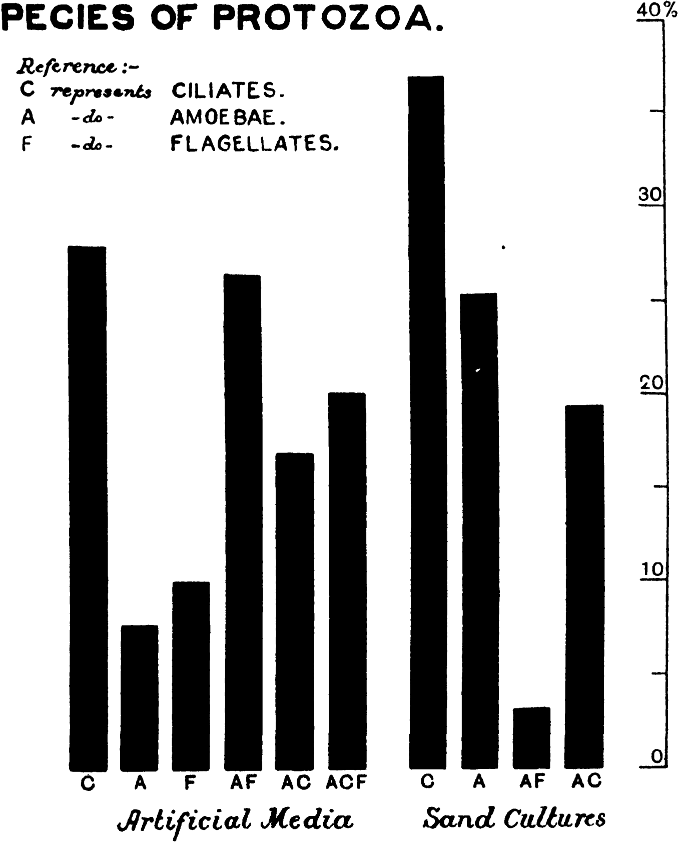
A perusal of the above results shows that the presence of protozoa has no depressing effect on the nitrogen-fixing bacteria, either in the artificial culture media, or in sand cultures. From a total of 36 experiments done in duplicates or triplicates, 31 showed a decided gain, while only 5 gave negative results. The average figure for fixation works out to be 8·5 per cent., which is well above the experimental error.

The highest fixation of 36·04 per cent. was recorded in sand cultures in the case of ciliates. All the three types of protozoa gave higher fixation figures. The experiment was repeated six times, and every time concordant results were obtained.

To make the results easily intelligible the highest figures, calculated as percentages, are shown in Diagram I. The vertical columns represent the percentage of nitrogen gained in presence of protozoa after deducting the figures for azotobacter and other bacteria.

At this stage of the experiment it is difficult to say in what manner the protozoa affect the increase of nitrogen-fixation above the value of azotobacter, whether they themselves take part in actual fixation, independent of the existence of azotobacter, or there is some symbiotic relation between the two types of organisms.

DIAGRAM I. SHOWING HIGHEST FIXATIONS RECORDED WITH DIFFERENT SPECIES OF PROTOZOA.



Richards and Sawyer's(11) experiments indicate the importance of protozoa in relation to the nitrogen content of activated sludge. They believe that a large proportion of nitrogen in activated sludge is due to the bodies of protozoa. In a crude mass culture of protozoa they found as much as 8 per cent. of nitrogen as compared with 7·5 per cent. contained in the richest sample of the activated sludge.

It is intended to extend these experiments so as to test these possibilities.

III.

A final experiment to confirm conclusively the previous results was performed by using a much larger quantity of media, the aim being to obtain bigger differences in the actual fixation values. The highest figure thus far obtained over the control was 2·86 mgms. of nitrogen, and it was thought that probably a large quantity of medium would give a higher value with the same experimental error as before.

The results are shown below:

Series VII. Effect of large quantity of media.

(Incubation period 15 days.)

No. of culture	Organisms* present	Mgms. of nitrogen fixed per gram of mannite	Gain or loss	
			Actual	Per cent.
80 c.c. *of media in* 500 c.c. *Erlenmeyer flasks:*				
14	C.N.†	15·75	+2·86	+22·19
7	A.F.N.	14·96	+2·07	+16·06
18	C.N. (pure culture)	13·25	+0·36	+ 2·79
10	N. (control)	12·89	—	—
100 c.c. *of media in large Petri dishes:*				
14	C.N.	24·06	+7·88	+48·7
7	A.F.N.	20·12	+3·94	+24·35
18	C.N. (pure culture)	18·9	+2·72	+16·81
10	N. (control)	16·18	—	—

* For detail of organisms please see Table I.

† C. means ciliates; A. amoebae; F. flagellates; and N. nitrogen-fixing bacteria.

Series VIII. Effect of large quantity of media.

(Incubation period 15 days.)

No. of culture	Organisms* present	Mgms. of nitrogen fixed per gram of mannite		Gain or loss	
		Parallels	Mean	Actual	Per cent.
50 c.c. *of media in large Petri dishes:*					
14	C.N.†	13·89 13·82	13·86	+3·12	+29·05
7	A.F.N.	11·96 11·89	11·92	+1·18	+10·99
18	C.N. (pure culture)	11·38 11·60	11·49	+0·75	+ 6·98
10	N. (control)	10·74 10·81 10·67	10·74	—	—

* For detail of organisms please see Table I.

† C. means ciliates; A. amoebae; F. flagellates; and N. nitrogen-fixing bacteria.

From the above tables it will be seen that large quantities of media have given bigger differences. The highest fixation is 7·88 mgms. in the case of ciliates, which means 48 per cent. net addition of nitrogen over the control experiment.

These figures not only confirm the previous results, but make one feel that the presence of protozoa plays an important part in the fixation of atmospheric nitrogen by azotobacter, and it will be interesting to continue this work with a view to discovering the factor or factors inducing these large fixations.

The work was carried out in Mr D. W. Cutler's laboratory, to whom I have a deep sense of gratitude for his ever-ready help and unfailing kindness. I greatly appreciate his invaluable suggestions and criticisms.

I am indebted to Sir John Russell for his kindness in giving me facilities to carry out the work in his institute, also for his unreserved courtesy, his sympathetic attitude and his keen interest in the work.

REFERENCES.

(1) CUNNINGHAM, A. (1914). *Journ. Agric. Sci.* VII, Pt. 1, 49–74.

(2) CUTLER, D. W. (1920). *Journ. Agric. Sci.* X, 135–143.

(3) CUTLER, D. W. and CRUMP, L. M. (1920). *Ann. Appl. Biol.* VII, 11–24.

(3 *a*) CUTLER, D. W., CRUMP, L. M. and SANDON, H. (1922). *Phil. Trans. Roy. Soc.* B. CCXI, 388.

(4) GOODEY, T. (1916). *Proc. Roy. Soc.* B. LXXXIX, 279–314.

(5) GREIG-SMITH, R. (1912). *Pro. Linn. Soc. N.S. Wales*, XXXVII, Pt. 4, 655–672.

(6) —— (1910). *Proc. Linn. Soc. N.S. Wales*, XXXV, Pt. 4, 808–822. Also Presidential Address. *Roy. Soc. N.S. Wales* (1916) and *Proc. Linn. Soc. N.S. Wales*, XXXIX, Pt. 3 (1914).

(7) HILL, T. L. (1916). *Journ. Bact. Baltimore*, I, 423–433.

(8) LIPMAN, J. G., BLAIR, A. W., OWEN, I. L. and MCLEAN, H. C. (1912). *N.J. Agric. Exp. Sta. Bull.* 248.

(9) MARTIN, C. H. and LEWIN, K. R. (1914). *Phil. Trans. Roy. Soc.* B, 205, 77–94. Also *Journ. Agric. Sci.* VII (1915), 106–119.

(10) MÜLLER, P. E. (1887). *Studien über die natürlichen Humusformen.* Berlin: Julius Springer.

(11) RICHARDS, E. H. and SAWYER, G. C. (1922). *Journ. Soc. Chem. Ind.* LXI, 5.

(12) RUSSELL, E. J. (1921). *Soil Conditions and Plant Growth*, 282.

(13) RUSSELL, E. J. and HUTCHINSON, H. B. (1909). *Journ. Agric. Sci.* III, 111–144. Also V (1913), 152–221.

(14) SHERMAN, J. M. (1916). *Journ. Bact. Baltimore*, I, 35–66. Also II, 165–185.

(15) WAKSMAN, SELMAN, A. (1916). *Soil Sci.* I, 135–152 and II, 363–376.

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OBSERVATIONS ON THE LIFE-HISTORIES OF *HYPODAERIUM CONOIDEUM* (BLOCH) AND *ECHINOSTOMUM REVOLUTUM* (FROEL): TREMATODE PARASITES OF THE DOMESTIC DUCK

By G. M. VEVERS, M.R.C.S., L.R.C.P., F.Z.S.

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(With 1 Text-figure.)

On May 8th, 1922, a number of encysted Trematodes were found in the liver of a *Limnea peregra* from Hendon, Middlesex. These were found to be larval Holostomes, probably *Tetracotyle typica* Diesing 1858.

This snail was fed to two Ducklings *A* and *B*, two days old, and on the 10th May another snail, also containing similar encysted Holostomes, was divided and fed to each Duckling.

On May 17th a *Limnea Stagnalis* from Italy was dissected and found to be infected with Rediae containing fully developed Echinostome cercariae. These were diagnosed as *Cercaria echinata* Sieb., the larval form of *Echinostomum revolutum* (Froel). The liver of this snail was fed to Duck *A*.

Operculate eggs measuring 100 × 60 were first seen in the faeces on May 28th (20 days after infection). On May 30th Duck *A* died and an examination of the intestine revealed the following parasites:

Fifteen specimens of *Hypodaerium conoideum* (Bloch) from the lower part of the small intestine.

Fifteen specimens of *Echinostomum revolutum* (Froel) from the cloaca.

The specimens of *Hypodaerium conoideum* were mature and the uterus contained fully grown eggs (22 days after infection).

The *Echinostomum revolutum* (12 days after infection) were immature. They measured on an average 4 mm. in length by ·75 mm. in breadth. The oral and ventral suckers were well formed and the head was surrounded by the typical collar of 37 spines. The cuticle of the ventral surface of the body between the head and ventral sucker was thickly covered with spines which gradually became fewer towards the posterior end of the body. The muscular pharynx and the alimentary caeca were fully developed.

The ovary, shell gland and yolk glands were in process of formation; and the uterus, which contained no eggs, was present as a thin-walled tube running forward from the ovary to the common genital pore, situated immediately in front of the ventral sucker.

The testes, which contained spermatozoa, were ovoid in shape and occupied a position one in front of the other in the posterior half of the body.

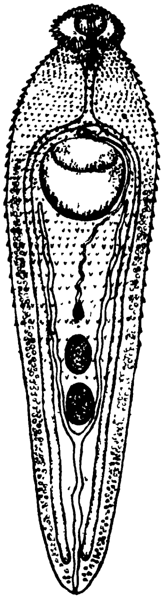

Fig. 1. Immature *Echinostomum revolutum* (Froel) from cloaca of Experimental Duck, 12 days after feeding with *Cercaria echinata* Sieb. (× 20)

The excretory system was **Y**-shaped, the bifurcation occurring between the posterior testes. Each branch of the **Y** could be traced forward as far as the ventral sucker. In more mature specimens the excretory system is not so evident. No Holostomes were recovered from Duck *A*.

On June 25th, 48 days after feeding with *Limnea peregra*, Duck *B* was killed; this bird, which had been passing fluke eggs since May 28th, was very thin and weak.

Forty specimens of *Hypodaerium conoideum* were recovered from low down in the small intestine. There was a localised inflammation of the mucous lining of the intestine over the area occupied by the flukes.

Although many encysted Tetracotyle were ingested by both ducks, no Holostomes were recovered from either. The specimens of Hypodaerium which were recovered from both ducks must have developed from some other cercariae also encysted in *Limnea peregra*, but not detected at the time of the examination of the snails.

CONCLUSIONS.

(1) That the intermediate host of *Hypodaerium conoideum* (Bloch) is *Limnea peregra*, the larval form being a cercaria, as yet undetermined, which encysts in some organ of that snail.

(2) That these flukes reach maturity in the small intestine of the duck in 20 days.

(3) That when present in large numbers they cause a considerable inflammatory reaction in the small intestine and may be responsible for a certain amount of debility and wasting.

(4) That *Echinostomum revolutum* (Froel) is Protandrous, the male sexual organs in the young form being in a more advanced stage of development than the female.

(5) That the encysted larval Holostome (*Tetracotyle typica* Diesing) from *Limnea peregra* does not develop in the domestic duck, or if it does, it produces adults so small as to be easily passed over at a post-mortem examination.

(*Received November 11th*, 1922.)

THE ACTION OF PROTOZOA ON BACTERIA WHEN INOCULATED INTO STERILE SOIL

By D. WARD CUTLER, M.A.

(*From the Protozoology Department, Rothamsted.*)

(With 1 Text-figure.)

It has been shown that in normal field soil the bacteria and active amoebae show an inverse relationship when their numbers are counted on successive days, and the conclusion has been drawn that the protozoa are in part responsible for inhibiting the normal increase in bacterial numbers[1]; but the further proof of this action, which ought to be obtained by inoculation experiments, has never been given.

Both in this country and abroad such proof has been sought, but with unsatisfactory results since in many cases there is no published evidence that the protozoa inoculated into soil survived in their new environment; while in others the countings were made at such long intervals as to preclude the possibility of deduction. Goodey[2], however, does record a case in which the bacterial numbers were reduced after the inoculation of amoebae cysts. He says, however, that there is no proof that excystation ever occurred and therefore does not connect the drop in numbers with the presence of protozoa.

In view then of the admitted crucial nature of such a test the following experiment was performed.

Ordinary field soil from a plot of ground annually manured with 14 tons of farmyard manure was sterilized in the steamer for one hour on four successive days. Tests showed that this had rendered the soil sterile. It was then divided into three portions each of 100 grms., which were placed in large sterile Petri dishes ready for inoculation. The inocula used were

(*a*) Bacteria alone.
(*b*) „ + one species of amoebae (*Dimastigamoeba gruberi*).
(*c*) „ + one species of flagellate (*Cercomonas crassicauda*).

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[1] *Phil. Trans. Roy. Soc.* B, vol. 211, pp. 317–350.

[2] *Proc. Roy. Soc.* B, vol. 89, pp. 297–314.

These were prepared as follows. Each of the species of protozoa had been so purified of bacteria that only three species remained, each of them being common soil bacilli. These were isolated free from protozoa and a suspension in sterile physiological saline prepared and counted in a counting apparatus. The two inocula of protozoa + bacteria were then made in similar suspensions so that each contained the same number of bacteria as did the control (protozoa free) suspension. In each case also most of the protozoa were in the encysted condition. This was important for previous experiments had shown that inoculations with active forms were rarely successful, the organisms usually dying soon after reaching the soil. Further it was found that inoculation by the addition of a given quantity of fluid to one portion of the soil was unsatisfactory, since often death of the active forms occurred; and also it took a considerable time for the protozoa and bacteria to spread uniformly through the soil sample.

In the present experiment therefore the inoculations were done by the use of a throat spray fitted with the finest nozzle obtainable[1].

By this means, however, it is difficult to record directly the size of the inoculations; so to ensure that approximately the same numbers of bacteria were added to each soil, the dishes and their contents were first weighed and then spraying the inocula was continued, until the same quantity by weight of fluid had been given, and the H_2O content of each soil had been brought to approximately 15 per cent.[2] Throughout the spraying the soils were shaken to ensure as far as possible uniform distribution.

Immediately after the completion of these operations counts by the plate method were made of both protozoa and bacteria; as will be seen by reference to the table the numbers of bacteria added to each soil were practically the same. The total numbers of protozoa added were:

Dimastigamoeba gruberi: 25,000 per gram.
Cercomonas crassicauda: 20,000 ,,

The soils were then incubated at a temperature of 20° C. For the first eight days after inoculation the numbers of bacteria were counted daily by taking from each dish 5 grms. of soil which was diluted to 1/1,000,000 in sterile physiological saline and plated out in the usual way. It was realised that 5 grms. was a small portion of soil to use, but this was the largest quantity practicable if the counts were to be long continued: moreover previous experiments had demonstrated that reasonably accurate comparative counts could be so made.

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[1] It may be mentioned here that this method has proved most satisfactory, and was used in the experiments detailed in a paper by Mr Nasir and published on p. 122 of this Journal.

[2] Every other day the soils were weighed and the water content brought to the 15 per cent. level by the addition of sterile tap water.

The protozoa for this first period were not counted. At the end of eight days counting was discontinued for a week, and then commenced once more for seven days. For this period the complete enumeration of the active and cystic protozoa was not done, but certain tests were made which demonstrated that these organisms were active in the soil, and on the fifteenth day the total numbers of protozoa were: *D. gruberi* 230,000 per gram; *C. crassicauda* 420,000 per gram.

The control soil throughout the experiment remained uncontaminated with protozoa.

In Table I and Fig. 1 the results of the experiment are set out.

Table I.

The numbers of bacteria are given in millions per gramme of soil.

Number of days after inoculation	Control (bacteria alone)	Control bacteria + amoebae	Control bacteria + flagellates
0	13·0	12·2	11·4
1	48·6	35·4	26·4
2	97·6	117·2	65·6
3	127·0	178·4	96·8
4	154·8	154·4	68·8
5	196·8	177·0	93·4
6	214·4	151·8	95·4
7	193·4	75·6	103·0
8	165·2	65·8	90·4
15	169·2	72·8	88·0
16	174·8	30·2	51·6
17	175·6	53·2	59·2
18	168·4	82·8	41·2
19	160·4	43·8	68·2
20	171·2	70·8	43·0
21	176·2	28·2	76·6

It will be noted that the numbers of bacteria in each of the soils rise steadily until they reach a maximum 6–8 days after the inoculations were made. This agrees with expectation since the bacteria multiply much more rapidly than do protozoa, which until their active forms are numerous are unable to exert any appreciable influence on the bacterial population. Also it will be remembered that the protozoa were inoculated as cysts, which, judging from analogy in fluid cultures, will probably remain as such for relatively long periods (24–28 hrs.) before excystation occurs.

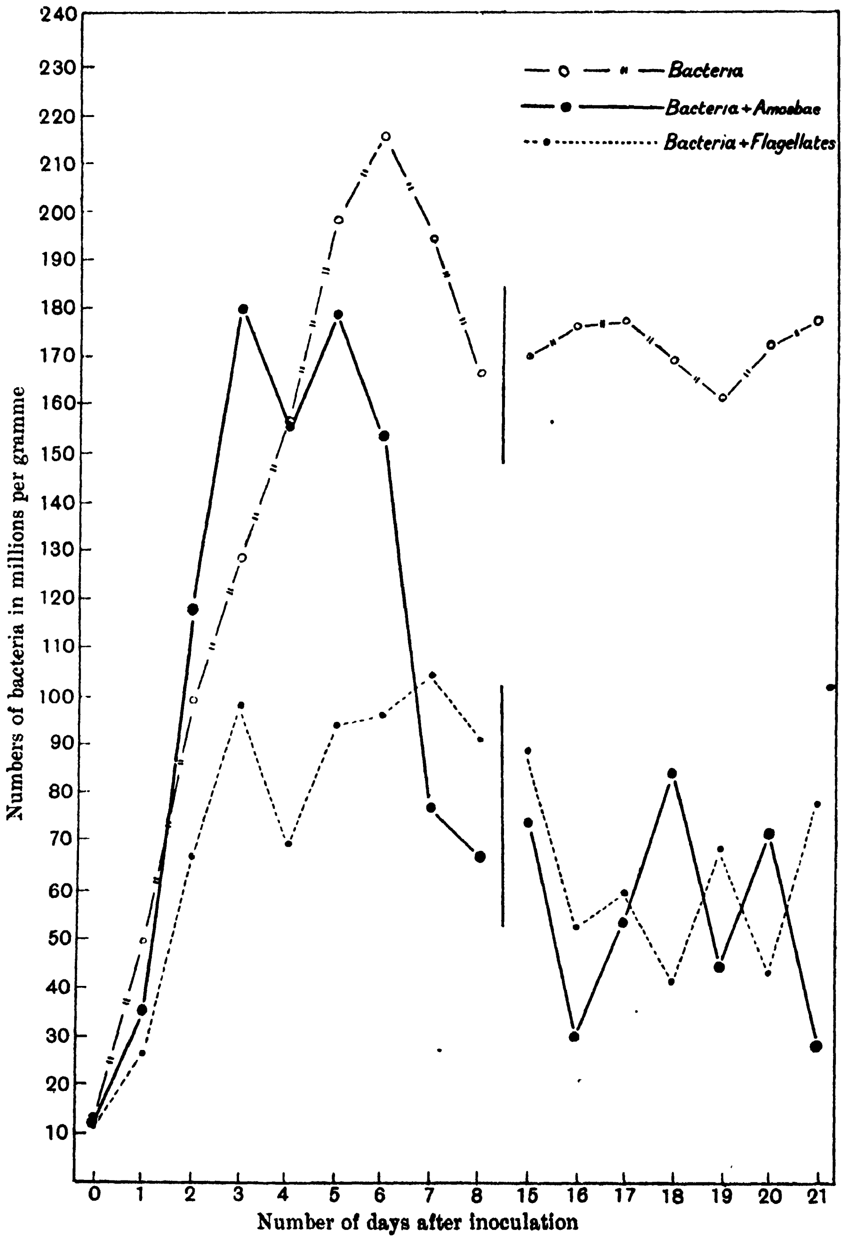
Bacteria
Bacteria + Amoebae
Bacteria + Flagellates
Numbers of bacteria in millions per gramme
240
230
220
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
1
2
3
4
5
6
7
8
15
16
17
18
19
20
21
Number of days after inoculation

Fig. 1.

In the case of the control and the bacteria + amoebae soils the final numbers attained are almost the same, 214·4 and 177·0 respectively; for some inexplicable reason, however, in the bacteria + flagellate soil the numbers of bacteria did not rise above 103·0 millions per gram.

The most interesting part of the experiment however, is the comparison of the bacterial numbers in the three sets of soils for the last seven counts, that is 15–21 days after inoculation. During this period the protozoa were known to be leading an active existence in the soil, and the relationship normally existing between them and the bacteria appears to have been attained.

In the soil containing only bacteria (control) the numbers for the last seven days of the experiment remained constant: the largest difference in two counts being only 11·2 millions per gram, which is not significant on the assumption that the error was 5 per cent.

In the other soils, on the other hand, the variations in the number of bacteria are considerable, especially as regards the soil containing amoebae, and are well outside the experimental error. Indeed the variations in numbers are comparable with those found from day to day in ordinary field soils under normal conditions and treatment. The soil containing flagellates (Cercomonas) does not show the fluctuations to the same extent, but again this is in accord with expectation, since Cercomonas, though an organism known to feed on bacteria, is not so voracious as *Dimastigamoeba gruberi*, the organism living in the soil containing amoebae.

Finally the experiment shows in a marked way that the bacteria in the soil free from protozoa are able to maintain their high numbers for a longer period than those living in soils containing protozoa.

In conclusion therefore it may be claimed that this experiment amply confirms the conclusion arrived that from other data, viz. that in normal field soil the bacteria and active amoebae show an inverse relationship: and that in such soils the presence of active protozoa are one of the factors concerned in keeping the numbers of bacteria below the level they might have otherwise attained.

(*Received November* 16*th*, 1922.)

STUDIES IN THE VARIETAL IMMUNITY OF POTATOES TO WART DISEASE (*SYNCHYTRIUM ENDOBIOTICUM* SCHILB., PERC.)

PART I

THE INFLUENCE OF THE FOLIAGE ON THE TUBER AS SHOWN BY GRAFTING

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INTRODUCTION.

THIS paper is an account of a first attempt to answer one of the many questions which arise when a biochemical study of the nature of varietal immunity to disease in plants is undertaken. The investigation is a section of the larger study of the problems of immunity to Wart Disease of potatoes and the control of this disease, in which the Department of Mycology and the Insecticides and Fungicides Department are collaborating.

It is well known that there are varieties of potatoes which have never been known to take Wart Disease; and the opinion has been held by pathologists for some time that this immunity is absolute, and not merely relative, as is the case in most plant diseases. Breeding experiments, notably those of Salaman and Lesley (1), tend to confirm this opinion. This apparently clear-cut distinction between "immunes" and "susceptibles" makes the case of Wart Disease of potatoes a good one for the biochemical study of the nature of varietal immunity to disease.

There is singularly little knowledge however of the general nature of this immunity. It may for example be conferred by a definite substance not present in the susceptible varieties, or on the other hand it may be due to the absence in the immune varieties of a substance which makes growth of *Synchytrium endobioticum* possible. If either of these suppositions be correct, this substance may be produced throughout the whole plant or it may be produced in one part and translocated thence to the remaining tissues. The possibility that the difference between "immunes" and "susceptibles" is the result of slightly different chemical groupings in the protoplasm in the two cases, does not seem to have been explored. Information of this nature is highly desirable before a biochemical study of the problem is undertaken; for each of the numerous possibilities, of which only a few are mentioned above, necessitates a different mode of attack.

Consideration of the functions of the various organs of the plant does not make one hypothesis more or less likely to be correct than another, nor does it help in the selection of any particular organ of the plant which is more likely to repay investigation than another. Mechanisms may be present whereby the root, the foliage and the stolon-tuber elements may be responsibie either singly or in conjunction with each other.

It was thought that light might be thrown on such questions and guidance obtained in the selection of the portion of the plant to be investigated first by exchanging elements of immune plants for corresponding ones from susceptible plants by means of grafting, and observing the effect on the tubers.

This paper deals only with the question whether the immunity or susceptibility of the tubers is in any way affected by the foliage which is responsible, in part at least, for synthetising the chemical compounds of which they are composed. The effect of the root, stolon and tuber part on the immunity or the susceptibility of the foliage, and the separate functions of the root, stolon and tuber elements are under investigation.

EXPERIMENTAL.

A healthy rapid growth of the plant was found to be an almost indispensable condition to success in grafting. The potatoes were grown in unglazed earthenware pots (11′ diameter) in a greenhouse, and a depth of 1″ to 2″ of sand was placed in the bottom of the pot to prevent water-logging of the soil. The pot was filled to within 1″ of the top with a mixture of equal parts of sand, finely sifted clay soil and finely sifted well decomposed farmyard manure; and the remaining inch filled with clean sand. The purpose of the latter was to prevent "caking" of the soil surface and excessive loss of moisture and to keep the potato stem clean. The pots were stood in saucers, and watering was only done when the leaves showed signs of withering in the hottest part of the day. The water was given in the saucers and the soil allowed to take it up slowly, thus avoiding the risk of water-logging.

Grafting was commenced when the plants were about 3″ to 4″ high, and the larger leaves were cut off to prevent wilting due to excessive evaporation from the scion. Plants were grafted usually in pairs that the "heads" might be simply exchanged; in this way both time and material were economised. After observing that the stems were clean, they were cut about 1″ above the sand, and the detached "heads" interchanged, care being taken to keep the cut surfaces clean. In each case the stem of the scion was sharpened into a wedge by two clean cuts, a slit made in the stem of the stock, and the wedge introduced so as to make a tight junction along the whole cut surfaces[1]. Success in grafting seemed to depend very largely on the speed and neatness with which these two last operations were carried out. The scion was tied in with artificial silk as tightly as possible without damaging the tissues unduly, and the joint was made air-tight by painting with paraffin wax kept molten on a steam bath. The whole plant was removed from the soil and all tubers larger than a sweet pea cut off; it was replaced in the pot, watered well from the top and placed in the shade, where it was allowed to remain until the scion was growing strongly. By observing these precautions it was found possible in most cases to prevent any appreciable loss of turgor in the scion, and when union was well established the paraffin wax and binding were removed.

Few plants were lost through infection at the graft; and this method was found to give better results than any in which the plants were disinfected. The tubers resulting from these grafts varied in size up to about 1½″ diameter. These were grown in infected soil, with the results shown below. In the table on p. 145 the composite plants are arranged in types according to whether the scion and stock are immune or susceptible.

It will be noticed that all tubers from types "immune on immune" and "susceptible on immune" give progeny free from Wart Disease, while all from types "immune on susceptible" and "susceptible on susceptible" give progeny which are infected. So thorough was the infection in the last two types that no tuber larger than 4 × 3 × 2 cms. escaped the disease and only a few smaller than this size were found clean. In most cases every eye of the tuber was infected.

Though the number of experiments was small these clear-cut results seem to justify the conclusion that in these experiments the immunity or susceptibility of the tubers arising from the stock is unaffected by the scion. The grafting of "susceptible" foliage on to an "immune" underground portion has not resulted in infection of a single "eye." There is no evidence of decreased infection in "immune on susceptible" type as compared with "susceptible on susceptible" type, as might be expected

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[1] A piece of "Gillette blade" soldered into a metal handle was found a very convenient grafting implement both because of its sharpness and its thinness, which avoided bruising of the cut tissues.

Results of susceptibility tests on tubers obtained from "composite plants."

Graft no.	Foliage / Below ground portion	Types	No. of Tubers obtained from composite plant	"Set" no.	No. of Tubers		No. of "Eyes"		
					Clean	Infected	Clean	Infected	
45	Arran Comrade	Immune	1		2	0	10	0	**Clean**
	Tinwald Perfection	Immune							
53	Kerr's Pink	Immune	2	(1)	4	0	16	0	**Clean**
	Arran Comrade	Immune		(2)	3	0	10	0	
54	Kerr's Pink	Immune	1		5	0	18	0	**Clean**
	Ally	Immune							
37	Arran Chief	Susceptible	1	(1)	3	0	16	0	**Clean**
	Ally	Immune		(2)	2	0	9	0	
40	King Edward	Susceptible	2		8	0	40	0	**Clean**
	Ally	Immune							
115	Up to Date	Susceptible	2		3	0	?	0	**Clean**
	Dargill Early	Immune							
30†	Great Scot	Immune	1		0	3	8	12	**Warted**
	King Edward	Susceptible							
39	Ally	Immune	1		4	7	17	23	**Warted**
	King Edward	Susceptible							
50	Arran Comrade	Immune	1		0	7	0	21	**Warted**
	Arran Chief	Susceptible							
57	Kerr's Pink	Immune	2		0	6	1	13*	**Warted**
	Arran Chief	Susceptible							
41	King Edward	Susceptible	1		0	8	4	19	**Warted**
	Arran Chief	Susceptible							
135*	Arran Chief	Susceptible	1		0	1	0	1*	**Warted**
	Alnwick Castle	Susceptible							

* Large masses of wart.

† In this experiment the old tuber was not removed. Seeing that a considerable amount of synthesis was to be expected from the quantity of foliage developed and that the young tuber seemed the only place where the products could be stored, the experiment was thought worth including.

to be the case if the "immune" scion had any influence on the "susceptible" stock.

Before leaving this work it is hoped to produce a few larger crops of tubers from grafted plants from which, on grafting, all stolons as well as tubers were removed; to increase the number of experiments; and to grow the actual grafted plant in infected soil and not the produce alone as in the above piece of work.

These results suggest that if immunity or susceptibility be due to a substance produced in one part of the plant and translocated thence to the other parts, then the foliage is not the source of this substance. Experiments are in hand to determine whether the below-ground portion has any such effect on the foliage, and these should indicate whether such a substance is produced in the former part. If it is, the effects of the separate units of the below-ground portion may be analysed further, whilst if there is no such effect, the hypothesis of some structural chemical difference between the products of the protoplasmic activities of "immunes" and "susceptibles" would seem worth testing.

Because of the smallness of the amount of data, this work is to be regarded as preliminary, but the fact that all the results are susceptible to one interpretation only seemed to warrant their publication.

It is a pleasure to express my indebtedness to Mr F. Tattersfield and Dr W. B. Brierley for much helpful criticism.

SUMMARY.

1. Grafting experiments of a preliminary nature have been carried out to throw light on the functions of the various organs of the potato plant in rendering the tubers immune or susceptible to Wart Disease (*Synchytrium endobioticum* Schilb., Perc.).

2. It is hoped that such knowledge may facilitate a biochemical study of the problem by indicating which part of the plant to investigate first, and to give some clue to the general nature of the chemical differences to be sought.

3. Composite plants were built up by grafting in the following ways:

3	plants	of the type	Immune	grafted on	Immune.
3	,,	,,	Susceptible	,,	,,
4	,,	,,	Immune	,,	Susceptible.
2	,,	,,	Susceptible	,,	,,

4. The results are in harmony with the conclusion that the character of the foliage has no influence on the immunity or the susceptibility of the tubers to Wart Disease.

5. It follows that no compound synthetised in the leaves is likely to be responsible for separating potatoes into "immunes" and "susceptibles."

6. The investigation is being continued, and it is hoped to determine whether any one part of the plant influences the immunity or susceptibility to the disease of any other part. The desirability of investigating a portion possessing such an influence is obvious. If the several parts of the plant have no such effect on each other, a search for differences in substances, such as the proteins, which are only translocated with difficulty, if at all, would be indicated.

REFERENCE.

(1) SALAMAN, R. N. and LESLEY, J. W. (1921). *Report of the International Potato Conference*, 105–111.

(*Received January 26th*, 1923.)

REVIEW

The Coccidae of Ceylon. By GREEN, E. E. London. Dulau & Co. 1922. Part V, pp. 345–472. Plates CXXXIII–CXCI *a*. £3.

The issue of the final part of *The Coccidae of Ceylon* marks the completion of the most important of the many works that have appeared on scale insects. The first part of Mr Green's treatise was published in 1896 and the others appeared successively in 1899, 1904, 1909 and 1922. The volume under review, like its predecessors, represents a very large amount of detailed labour requiring close application with the microscope. Its value is greatly enhanced by the profusion of plates illustrating almost every important structural point in the species dealt with. The excellence of these plates will commend itself to general admiration but, good as these illustrations are, they are excelled by the author's original drawings. It is a matter for regret that few, if any, methods of illustration are capable of reproducing natural objects with the identical fidelity and delicacy of the human hand.

The island of Ceylon represents a limited area, nevertheless its fauna is one of tropical richness, and nearly 300 species of Coccidae have been recorded from within its limits. A number of the species, it is true, are cosmopolitan or nearly so, but the total Coccid fauna of Ceylon includes a very considerable percentage of that of the world. A work of this kind is particularly valuable to Indian entomologists, not only in view of the great economic importance of the family concerned, but also from the fact that the Indian Coccidae are much less known and have not, so far, been comprehensively dealt with.

It is inevitable in a monograph whose publication has extended over 26 years that radical nomenclatorial and other changes have resulted in the meantime: these fortunately are dealt with in an appendix at the end of this volume. We can sympathise with the author in his reluctance to discard such familiar names as *Coccus* for the cochineal scales and *Lecanium* for the brown scales in conformity with the law of priority. These and other changes are particularly confusing to the non-specialist since, in several cases, the discarded names have still to be retained although in connection with totally different genera.

Among features of the present volume is the occurrence of only a single species (*Kuwania zeylanica*) of Margarodinae in Ceylon. Three species of *Margarodes* are found in S. India, but none so far have been detected in the island fauna. Similarly, *Tachardia lacca*, which has a wide range in India and Burma, is not indigenous to Ceylon. This discontinuity in certain elements of faunae of the two regions is well known to be borne out among the larger animals also. Among insects it is perhaps not always a real one, and the discovery of forms such as *Margarodes* in Ceylon is within the bounds of probability.

In conclusion, it may be said that all entomologists will congratulate Mr Green upon the completion of his laborious task. The uniform excellence of the plates, which number 209 in the whole work, is in itself a great achievement. It may also be added that the manner in which these plates are reproduced is no mean tribute to Dutch lithography.

A. D. I.

REPORT OF THE COUNCIL

OF THE ASSOCIATION OF ECONOMIC BIOLOGISTS FOR THE YEAR 1922-3. PRESENTED TO THE ANNUAL GENERAL MEETING, JANUARY 26TH, 1923.

DURING the year seven meetings have been held which have usually been devoted to discussions. The meetings have been well attended, an average of 64 members and guests being present.

A Field Meeting was held during the summer when the Association met at the Royal Horticultural Society's garden, Wisley, at the invitation of the Director, Mr F. J. Chittenden, and the work in progress was demonstrated by the Director and his colleagues.

Reversion to a previous custom was made in the holding of a provincial meeting at Manchester on Dec. 15th and 16th, the local arrangements being carried out by Dr Wilfrid Robinson. The afternoon and early evening were devoted to communications; members were entertained to tea by Professor F. E. Weiss and his colleagues, and an Association Dinner was held in the evening. On the second day the Shirley Institute of the British Cotton Industry Research Association was visited, members being welcomed by the Director, Dr Crossley, and having the work in progress demonstrated to them by the several heads of departments.

During the year the Association sustained a great loss by the death of Mr Bacot, a member of your Council, and an obituary notice was printed in the *Annals of Applied Biology*. Professor J. H. Priestley was invited to fill the vacancy. Since the last Annual General Meeting eleven candidates have been admitted to ordinary membership of the Association and the number of members exclusive of those whose subscription is three or more years in arrear now stands at 234, a decrease of 6 as compared with last year.

Your Council considers that the scientific progress of the Association has been well maintained during the year, but wishes to urge the desirability of introducing as members of the Association additional active workers who may assist in its development.

The thanks of the Association are due to Professor J. B. Farmer and his colleagues for granting the use of rooms for the meetings of your Council, and, further, for their unfailing kindness and hospitality in permitting the Association to meet in the Botanical Lecture Theatre of the Imperial College of Science.

VOLUME X JULY, 1923 No. 2

I. THE TURNIP GALL WEEVIL. *CEUTHORRHYNCHUS PLEUROSTIGMA*, MARSH. (*COLEOPTERA, CURCULIONIDAE*)[1]

PART I. LIFE-HISTORY AND BIONOMICS

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(With Plate V and 12 Text-figures.)

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INTRODUCTION.

THIS paper embodies certain observations on the life-history and habits of the Turnip Gall Weevil. The investigation was started in August, 1919, at the suggestion of Prof. H. M. Lefroy. A second paper on the anatomy of the larva of this weevil has also been prepared. Work on the metamorphosis and control experiments is in progress. I have to express my gratitude to Prof. Lefroy under whose direction the work is being conducted, and my thanks to Mr J. C. F. Fryer, Entomologist to the Ministry of Agriculture, for valuable suggestions, and to Messrs P. Cragg and W. J. Lobjoit for giving me all facilities on their farms to study the pest and also to the Ministry of Agriculture for a grant towards the expenses of the research.

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[1] A grant in aid of publication of this communication has been given by the University of London.

CLASSIFICATION AND DESCRIPTION.

The Turnip Gall Weevil, *Ceuthorrhynchus Pleurostigma* Marsh (*sulcicollis* Gyll) belongs to the family Curculionidae in the order Coleoptera. The specific name is based on Fowler's *Coleoptera of the British Isles* (3).

A full technical description of the species is to be found in Curtis's *Farm Insects* (2).

METHOD AND RANGE OF ENQUIRY.

The observations recorded here are the results of laboratory and field-work, the former carried on at the Imperial College of Science, South Kensington, and the latter on farms around Osterley Park, Middlesex.

The habits of the pest and the liability to attack of the various host plants, both wild and cultivated, were studied in the field. For general study of the insect, adults were collected from the field and kept in cages outside the laboratory so that they were under very nearly natural conditions. These beetles were found to feed quite well on fresh turnip slices. Galled plants collected in autumn were kept in cages, some exposed to natural conditions and others protected from rain with the soil either quite dry or with only enough moisture around the rootlets to keep the plant healthy. These gave particulars as to development and the influence of climatic conditions.

The minute details of fertilisation, egg-laying and fecundity were obtained by caging under field conditions, imagines reared in field cages and of known history. These were kept either in numbers or when necessary in couples after observation of the first pairing. Young cabbage plants daily supplied to the latter were regularly examined under a binocular microscope for eggs.

Owing to the extreme difficulty of finding the egg, the unlikelihood of an exposed egg hatching and the impracticability of keeping a particular larva under observation while developing without upsetting normal growth, no individual life-cycle could be followed completely through. The necessary facts of the life-cycle are therefore gathered from observations on a large number of individuals in various stages of growth through partial periods of development.

DISTRIBUTION AND HISTORY.

This weevil has been reported as assuming pest proportions in several countries in Europe, and occurs all over the British Isles.

The earliest record of an entomological nature of this insect is in Kirby and Spence's *Introduction to Entomology*, First edition, 1815(6), where it is stated that it must be certain weevil larvae "that cause the clubbing of the roots of cabbages and the knot-like galls on turnips." In a later edition of the same work dated 1856(7), Spence says that he reared out *Curculio pleurostigma* Marsh (now known as *Ceuthorrhynchus pleurostigma*) from the galls on turnips.

Curtis(2), writing in 1859, included this beetle in his *Farm Insects* but did not think they did more damage than affecting the beauty and symmetry of turnip bulbs. Later, Miss E. A. Ormerod(12), in 1881, wrote that the galls may often run together and exhaust the strength of the plant, and Prof. Theobald(15) in 1907, in *The Report on Economic Zoology*, considered the pest as causing incalculable damage all over the country in turnips and swedes.

HOST PLANTS.

During the course of the present investigation various cultivated *Cruciferae* which showed galls were kept under observation. These included the different varieties of *Brassica oleracea*, as cabbage, cauliflower, kale, broccoli and brussels-sprouts, and of *B. campestris*, as turnips and swedes. In every case this one species of weevil was found to be the cause. *Sinapis arvensis* (charlock) is a favourite wild food-plant. Galls have not been noted on other wild crucifers, though many of them, especially those that occur as weeds on farms, were carefully examined.

ECONOMIC IMPORTANCE.

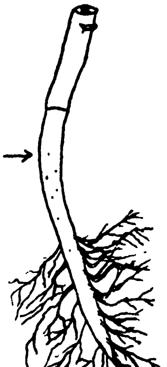
The larvae of the gall-weevil are found below the bark on the root of various cruciferous plants. Their presence checks the natural growth of the host plant and induces it to form galls. Older plants may, apparently, remain vigorous, but when young plants are attacked they become stunted and unhealthy or completely fail to grow. The larva feeds mainly on the cortical cells, and the growth of the gall supplies it with plenty of such material. But the abnormal process of producing the gall must in itself be detrimental to the vitality of the plant. In very young plants there may be present several larvae which on account of the small size of the galls will bite into the wood, thereby destroying a large amount of meristematic and fibro-vascular tissues and injuring the plant beyond recovery.

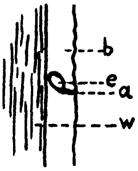
SUMMARY OF LIFE-HISTORY.

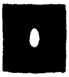
The female weevil lays her eggs singly in the bark on the tap-root, bulb or underground portion of the stem of the selected host plant. A legless grub hatches out and feeds on the inner bark. The presence of the larva stimulates the plant to form a gall around the injured spot. The grub lives inside the gall until full-fed and then bores out into the soil and pupates in an earthen cocoon. From the pupa the adult develops and crawls up to the surface to feed and reproduce.

DESCRIPTION OF VARIOUS STAGES.

The egg (Text-figs. 1, 2 and 3) is minute, almost transparent and soft, oval in shape and measures 0·35 mm. in length and 0·25 mm. in breadth. But these measurements may vary slightly and the egg may appear more roundish. The female always lays her egg in a cavity specially drilled in the bark for the purpose, and of a size just large enough to allow the egg to fit in tight. The soft egg thus held under slight pressure adjusts itself to the shape of the cavity.







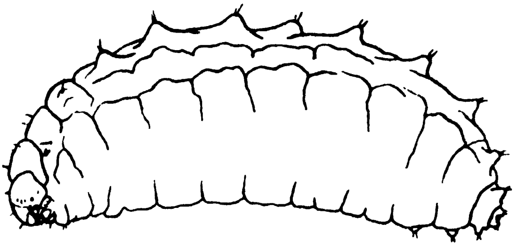
Text-fig. 1. Root-portion of young cabbage plant; the arrow marks ground-level; the dotted area marks region favoured for oviposition. (½ nat. size.)
Text-fig. 2. Egg *in situ*. (× 8.) *a*, puncture; *b*, bark; *e*, egg; *w*, wood.
Text-fig. 3. Egg. (× 8.)

The larva (Text-fig. 4) is typically curculionid in form, whitish and when full grown about 4 mm. long. The head is small but well exserted.

The mouth parts are hypognathous. Three thoracic and ten abdominal segments are distinct, the last abdominal segment being very minute.

Except for a small fleshy labial area the cephalic region is entirely covered by a well chitinised head-shield on which dorsally the epicranial suture, dividing off anteriorly into the frontal sutures, is clearly marked. The antennae are only represented each by a short two-jointed process close to the base of the mandible at the lateral angle of the frons. There is a distinct dark eye-spot beneath the exoskeleton, on either side.

The body is very much wrinkled transversely. The prothorax has a dorsal thickening into which the head may be slightly withdrawn. The cuticle on this area is minutely punctate. There is a lateral row of tubercles, one on each segment, those on the last abdominal segments being very pronounced, but the others dwindle as they near the head, and there is no tubercle on the prothorax.



Text-fig. 4. Larva of *C. pleurostigma* Marsh, in third instar. Ventro-lateral view showing lateral tubercles. (× 15.)

Spiracles are present on the mesothorax and on the first eight abdominal segments. There are a few minute spines distributed over the body, those on the head, the prothorax and the lateral tubercles being more prominent.

The grubs are usually whitish in colour, but, being rather transparent, those that feed in swedes, owing to the contents of the intestines, may appear reddish-yellow.

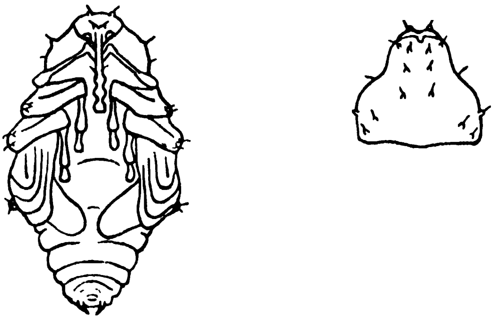
The larva moults three times before pupation, and the three instars are separately described below.

First instar. The just-hatched larva is 0·7 mm. long and 0·2 mm. across the head-shield which has in this instar a grey colour.

Second instar. Directly after the first moult the larva is 0·9 mm. long and 0·35 mm. across the head-shield. The whole body as well as the head appears remarkably translucent.

Final instar. At the beginning the larva is 2 mm. long and 0·63 mm. across the head-shield, which is distinctly brownish-yellow and hard in this instar. When ready to pupate the length is nearly 5 mm.

The pupa (Text-figs. 5 and 6) is uniformly white when just transformed and measures 3·7 mm. in length and 2·5 mm. across the broadest part of the body. The head is withdrawn into the prothorax and the rostrum folded on its venter. The antennal scapes run cephalad along the sides of the rostrum, the funicles are directed outward and caudad. Pro- and meso-thoracic legs are folded on the venter and lie over the wing sheaths which are directed on to and press close on the venter. The elytra cover the basal portions of the lower pair, whose exposed distal tips meet or nearly meet on the venter of the abdomen. The third pair of legs are folded below the wings and only the femero-tibial joints of each is exposed, being visible on the lateral aspect of the pupa. Looked at dorsally, the head is only seen to a small extent as it is withdrawn into the collar-like anterior margin of the prothorax. Scattered over the body there are several tubercles bearing short stiff chitinous bristles. These serve to support the pupa in the cocoon, keeping the soft body from contact with the earthen covering. There is one pair of such spines on the vertex, one pair between the eyes, one pair about the middle of the rostrum and one pair on every femero-tibial joint. On the dorsal aspect of the pronotum on either side of a median groove there is a longitudinal row of three and laterad of these, one close to the anterior margin, one where the pronotum broadens out, and a group of three on the prominence at the hind angles of the pronotum. At the posterior extremity of the abdomen there is a pair of very strong terminal hook-like spines.



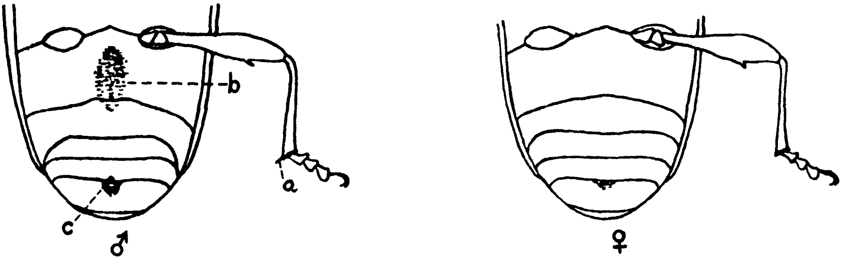
Text-fig. 5. Pupa of *C. pleurostigma* Marsh, ventral view. (× 10.)
Text-fig. 6. Dorsal view of head and prothorax of pupa. (× 10.)

As development proceeds the dark compound eyes become manifest, and within their respective sheaths the antennal scapes, the claws and the wings form and darken.

The adult. The size of the individual does not vary to any marked extent.

SECONDARY SEXUAL CHARACTERS.

Certain minute external characters which differentiate the sexes may, however, be dealt with here (Text-figs. 7 and 8). The male has a strong spine at the distal extremity of the hind tibiae and a widely impressed median neutral area on the abdomen occupying the apparent first and part of the second sternite; also on the fourth abdominal sternite on its posterior margin a minute median indentation with a short tooth on either side of it. Examination of the abdominal tergite shows that while in the female the pygidial position is occupied by the seventh, in the male the seventh tergite is pro-pygidial, an eighth visible tergite being the pygidial. It may be stated here that the use of the terms pygidium and pro-pygidium, as is often done, without mentioning the numerical relation of the segment, would be confusing. The seventh tergite of the female is thickly chitinised and granular. In the male the seventh and part of the eighth are uniformly thickened and granulate and fit together to occupy an area corresponding to that of the seventh alone in the female.



Text-figs. 7 and 8. Ventral view (diagrammatic) of abdomen and left hind leg of male (Fig. 7) and female (Fig. 8), to show sexual difference (much magnified). *a*, tibial spine; *b*, impressed area on abdomen; *c*, indentation on fourth sternite.

BIONOMICS.

Emergence of adult. The adult beetle emerges from the cocoon by bursting it. By spreading out the legs and raising its body the beetle secures a firm wedged-in position inside the cocoon. The rostrum, which has till now been more or less closely applied to the venter of the thorax, is now forced outward, causing it to press against the cocoon, until it splits.

This is the normal method of emergence and is only possible under normal conditions when the cell is fairly moist and yielding. But if cocoons are kept dry they become so hard that a large proportion of beetles fail to break through, though they may live for nearly forty days without food in the cocoon. The few that emerge only succeed owing to some accidental flaw in their cocoons.

Pupation takes place among the roots and therefore cocoons are usually found from the surface to about four inches below. Where they were experimentally buried eight inches below soil the imagines emerged.

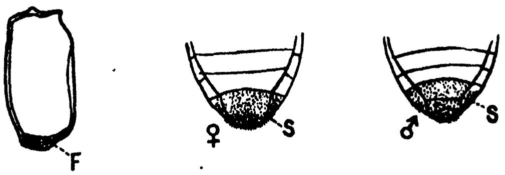
Directly the beetles come to the surface they seek and take shelter in some dark place under clods, or fallen leaves, or in a crevice in the soil. They will remain here for about 24 hours, attaining the final degree of hardness before they wander about.

Protective habits. These weevils are well adapted to protect themselves from their enemies. Being small and uniformly black dorsally, it is very difficult when they are in the soil to discriminate them. Further, they are very slow, seldom take to flight and generally remain hidden while feeding, resting or travelling. If they happen to be in an exposed position, at the least perception of danger they will relax their hold and drop down. A quickly moving object within a few inches of them will make them do this.

After dropping down and thereby temporarily escaping from danger, it ensures greater safety by turning on its back as soon as it comes to rest, folding the legs close to the body and remaining perfectly motionless. In this attitude the yellowish-grey colour and scaly appearance of the ventral surface, the roughened flattish aspect and the different outline, contribute to produce a picture quite different from the dorsal view of the insect. By remaining motionless the similitude to an irregular particle of some lifeless material is completed. This procedure is usually known as "shamming or feigning death," but it should be understood that it works on the lines of mimicry and that if an insect only shammed to be dead but continued to present any degree of resemblance to its original figure it does not augment the chances of its safety.

Still another means of protection is the power to frighten enemies by producing a quick succession of short screeches.

Stridulation. The sound is produced by protruding and retracting the abdomen rapidly, thereby drawing the apical hard granulate dorsal surface, provided by the seventh tergite in the female and the seventh



Text-figs. 9, 10 and 11. Stridulating mechanism; Fig. 9, ventral view of left elytron; Fig. 10, granulated area ("scraper") in female; Fig. 11, the scraper in the male. (× 12.). *F*, file; *S*, scraper.

and eighth in the male, across the minute transverse ridges on the flattened area on the inside of the apical margin of the elytra (Text-figs. 9, 10 and 11).

Both sexes can stridulate, and it has often been observed that when a beetle is picked up between the fingers it will immediately try to produce the squeak. The sound is quite audible when the insect is held close to one's ears. The only other occasion when this sound is produced is when copulation is attempted and then only by the male. After mounting a female the male in trying to copulate throws out and retracts its abdomen a number of times. The sound produced may probably exert some sort of sexual attraction on the female. But as both sexes invariably stridulate when suddenly picked up, it is probable that the function of the sound is mainly protective.

Food habits. The adults feed on leaves, tender stems, the bark on the root, young pods and flowers of turnips and cabbages and on the foliage and flowers of charlock (*Sinapis arvensis*), hedge-mustard (*Sisymbrium officinale*) and a plant allied to the water rocket (*Radicula palustris*).

As mentioned before, the beetles hide away for about 24 hours from the time of emergence and then begin to feed, always trying to remain concealed. In feeding they bite a hole into the substance and then scoop out as much of the inner succulent tissues as may be reached by twisting the rostrum about. In trying to hide they seem to feel safe only when they are tightly wedged in, away from light either in the soil or within curled up leaves. When about 15 days old they become very active for a period of three or four days and climb up plants, specially on a sunny afternoon, to feed on the inflorescence. Next, they conceal themselves in the soil for about two months, living apparently on little or no food. Sexual maturity is developed during this stage in beetles which emerge early in the year. But those that emerge later reach sexual maturity only after passing the following winter. This statement will be made clear when the different broods are dealt with.

The next phase in the life-history is chiefly concerned with reproduction. The beetles, after the period of concealment or hibernation, resume activity, feeding voraciously, specially on leaves and roots of crucifers, the roots of suitable host plants being specially attractive to the female. They copulate repeatedly, either in the soil or on the bushes, often exposing themselves, but usually when above the soil, keeping to the under surface of the leaves, particularly on the margin as the female often goes here for food, carrying or followed by the male.

Age at the beginning of fertilisation. These beetles are not sexually mature at the time of emergence, and a long time has to elapse before they exhibit reproductive impulses. Females that emerge early in the year, dissected periodically for six weeks from the time of emergence, showed no formation of eggs. Individuals nine weeks old attempted to copulate when a number of them were confined in a small cage. But in nature generally mating starts only a little later, and in the case of the later brood mating starts only after hibernation.

Fertilisation. Mating takes place on the bushes and in the soil, and the male may remain mounted on the female and be carried about by her for about twelve hours; the actual copulation, however, occupies only a few minutes. As is often the case with weevils, copulation is often repeated during the active life of the individuals.

Age at beginning of oviposition. At least five days have to elapse from the time of the first mating before eggs are laid.

Place of oviposition. The egg is always laid singly in the bark of the tap root or bulb of succulent healthy plants over six weeks old. The number of eggs laid in one plant is only limited by the amount of suitable surface available.

Selection of plant for oviposition. Soft, succulent, healthy, easily penetrable bark tissue is what the egg-laying female tries to select for oviposition, and in this respect the bark on the underground root certainly offers most advantages. Among the roots the rapidly swelling tuber of turnips is the favourite, next comes young cabbage plants and then the various other cultivated crucifers. As breeding proceeds and suitable places become rare, freshly formed galls and the bark at the collar region may all have to receive eggs.

Act of oviposition (Plate V, figs. 1 and 2). The female crawls down the stem on to the root and drilling into the bark with its rostrum excavates in the inner bark a small oval chamber of dimensions slightly less than those of the egg. She withdraws her beak now, and turns about, bringing the abdomen over the puncture. By means of the ovipositor the egg is thrust in. With the egg there is also secreted a gummy substance which quickly hardens and forms a covering at the mouth of the chamber protecting and keeping healthy both the egg and the lacerated plant cells.

The method of oviposition is necessary because the egg has to be protected from adverse conditions and provision has to be made for the helpless, just-hatched larva, to find food easily.

Fecundity. A female beetle on dissection shows four egg-tubes. One which had just started ovipositing had 12 well-developed eggs and in each tube a series of eggs in course of formation. An egg-laying female will lay per day four eggs during the first three or four days, two during the next few days and only one egg later on. After the first month egg-laying becomes irregular. Sunny evenings and warm nights seem to favour oviposition, no eggs being laid when the days are cloudy or rainy. A study of caged pairs leads to the conclusion that a female may lay a total of 60 eggs.

Duration of egg-stage. The egg hatches in five to seven days in summer and in about 17 days in autumn. Eggs laid very late in the year may hibernate. Some of the autumn eggs may hatch when 60 or 70 days old during a spell of warm weather.

Hatching. The egg is nearly transparent when laid, but when about to hatch, the grey head may be made out occupying one end of it.

It has been said that if the egg is laid loose the larva would be unable to work its way out. This is due to the membranous egg covering and the limitations on the working of the larval mandibles which is the only means the larva has of hatching. As pointed out by Prof. H. M. Lefroy (10) in the case of the larva of *Xestobium tessellatum*, the mandibles open out and meet like the two teeth in a nail-extracting pincers. If the surface to be worked into is plane the two teeth will only slip along on closing and will not get a bite. But if with the teeth apart the head can be pressed into the surface the teeth sink a little into the material and can close into a grip. The larva is curled up in a crescentic form inside the egg. By contracting the abdomen and fixing its tip to a point on the egg shell nearer the head the turgidity of the body is increased and considerable pressure acts on the head, pressing it outward. This may be still better explained by considering the effect of the above movements on the body fluid which is normally under high pressure. The contraction of the abdomen displaces from within it and drives into the anterior region a certain amount of fluid. The abdomen has been so pulled up and fixed that it cannot expand and occupy its original position. The body fluid thus made to occupy lesser space than usual will exert great pressure on the body-wall.

The egg-membrane offers another difficulty. Being soft it will behave like a thin sheet of elastic spread on a frame and will stretch and recede when the mandibles try to sink into it. But if the membrane is closely applied to a fairly hard, non-elastic surface then its elasticity has no play and the mandibles can secure a grip. This explains why the mother beetle leaves her egg under a certain amount of pressure.

The larva. The larva has already been described, and it has been stated that it passes through three instars.

How larva moves and feeds. Each larva gives rise to a gall within which it remains feeding on the cortical tissues, seldom biting into the wood. As the gall grows rapidly the chamber inside it, which the larva makes by eating, does not appear very large. In trying to bite off tissues the larva adopts the same method as in hatching out. Having so fixed its abdomen that in its curved position it can get its downwardly directed mouth parts pressed against the plant surface, it will begin to gnaw off tissues, making first a small cavity and then extend it downward into a groove, the greater curving of the body required to do this maintaining the original extra turgidity and thus saving the larva from having constantly to alter its position.

In locomotion also the device is about the same, the lateral tubercles on the body being here of immense help as they serve to keep the body fixed at points other than the anal tip. To make a forward movement the posterior region of the abdomen is contracted and fixed by means of the tip and the tubercles against the wall of the larval chamber and then the contractor muscles are relaxed. Both these actions take place very quickly. Hereby the body-fluid is subjected to high pressure in a reduced body cavity and then allowed to hammer back, upon which it distends the abdomen and, not having spent all its force, sends a pulsation along the line of least resistance which is towards the head. As it passes along the pulsation pushes each segment a little forward, and when it has pushed the head also the body comes to the normal state of expansion and the body-fluid to its usual state of turgidity and equilibrium is restored. In a distending larva it is possible to see one of the middle segments having its posterior region pressed forward by the expanding segment next behind it, and when the pulsation passes into it, itself expanding towards the anterior and then coming to rest. The grub by this contrivance is able to move every time a length equal to the distance it pulls up its abdominal end. It is possible for it to move backward by reversing the process and contracting the anterior thoracic segments first.

The gall. The gall, though separate for each grub, may often occur in clusters and may then coalesce to some extent. A single gall is a smooth rounded knobby growth about 10 mm. across at its base. In a cluster apparently made of four or five galls 10 to 15 grubs may be found, each in its chamber. While in the feeding stage the larva never bites its way close to the epidermis.

The gall in this case is due to some stimulus acting on the cells next to the larva and causing an abundant and rapid formation of cortical cells. According to Kerner's (5) classification of galls, since only a single plant organ is affected in this case and the gall has no natural cavity or opening, it is to be regarded as one of the "simple solid" ones.

As in the case of most galls which arise from ground tissues, the peculiar appearance is brought about by the abnormal quantitative increase in the number of cells in a definite region—which process Küster (9) terms hyperplasia as different from hypertrophy where the individual cells become modified in size and appearance and which is the way most epidermal galls are formed.

The actual stimulant that causes the hyperplasia is not known, and the investigation into its nature affords a delicate and difficult problem.

Pupation (Plate V, fig. 3). After the larva is full fed it waits for a short time, when its body swells very much and becomes full of a clear liquid. On dissection it is found that this appearance is due to the Malpighian tubules becoming distended with a clear secretion. A larva which has reached full growth will leave the gall only when the days are warm, reaching a maximum temperature of over 56° F., and the soil around has received a good supply of rain, and is quite moist. If galled plants are pulled out and kept dry the larvae will remain within and dry up with them. When under artificial conditions the soil outside is kept dry the larva will prepare its exit, leaving only a thin bit of epidermis intact so that it can emerge at the earliest opportunity, and will retire into the gall. But it will not pupate within. In a stalk, left in the field to dry, the larva will show signs of life for several days up to about eight weeks. When infested stalks are pulled out and spread about in the open after every shower of rain several larvae may emerge and this may continue for about 20 days, after which period the bark dries too hard for the larva to bite through. In breeding cages it has been noticed that grubs ready to cocoon if thrown into dry soil will not cocoon but will die in about 20 days, and that if fresh cocoons are kept dry the pupae may form but will die before developing into the imago.

The cocoon (Plate V, fig. 4). As soon as they reach the soil the grubs make a cocoon of mud and a gummy secretion. The cocoons are found close to the roots and sometimes close to the surface.

The gum used by weevil grubs in cocooning has usually been supposed to be a fluid emitted from an opening just above the anus. Recently, F. Knab (8) stated that the fluid is mainly the product of the Malpighian tubules voided through the anus and that certain rectal and surface glands may also contribute. In the present case the fluid is entirely the product of the Malpighian tubules, which, it has been previously stated, become, before pupation, swollen with a liquid content.

In about six hours from emergence the cocoon is constructed, though some more time is spent in smoothing down the inside. The larva on finding a spot with loose soil secretes a thin clear fluid from the anus and by wriggling and turning about moistens a layer of soil particles around it. The fluid being gummy holds these particles together. Then the larva curves ventrally to the utmost, bringing the head and anal ends together over a minute pellet of earth, moistens it well with the secretion, picks it up between the mandibles and straightening itself fixes it somewhere in the wall around it or where it finds strengthening necessary. The excess fluid is plastered on the inside by spreading it with the frontal region of the head. This goes on until the gummy soil forms around the larva a uniform crust, with a smooth inner surface well covered with a layer of gum. When the gum dries it causes the soil forming the cocoon to hold together more tightly and also forms a brown leathery lining inside.

After completion of the cocoon metamorphosis proceeds, the larva becomes inactive, the eye spots fade and the thoracic region swells, showing distinct conical areas marking the adult's legs.

The larva now moults and the pupal form appears. The skin splits transversely behind the head and from this another split runs caudad along the dorsum. The head-shield splits dorsally along the sutures into two lateral and one anterior piece which are held together by the cuticle on the fleshy labium and carries with it the old chitin of the mouth-parts. The whole cast covering is rolled off in one piece along the venter towards the anal end.

The pupa rests in the cocoon by means of the spines mentioned in the description. For the proper development of the pupa the cocoon has to remain slightly humid. If it gets dry or soaked the pupa will die. The mode of emergence of the adult has been dealt with already.

DURATION OF LIFE OF ADULTS.

Since the weevil continues to oviposit for several days the question of longevity is of decided importance. Adults that emerge in early summer from hibernated larvae continue active throughout the summer and autumn. All these die off in winter.

Beetles which emerge in late summer often live through the winter and the following spring. The length of imaginal life therefore seems to be nearly seven months when there is no hibernation and about nine months in case the adult hibernates.

HIBERNATION.

The eggs hatch soon after they are laid, but a few laid in late autumn may hibernate and hatch when there is a succession of warm days. Larvae become inactive when temperature falls below 50° F. All the larvae from eggs laid from the last week of August up to the end of the year will hibernate inside the galls, pass the winter and according to the growth they had reached emerge early or late in spring. No pupae have been noted in winter. The adults are active up to 45° F. and hibernate below that temperature.

LIFE CYCLE.

Egg. The egg hatches in summer on the fifth to the seventh day after it was laid, but takes longer in cold weather.

Larva. The period spent in the different instars is very variable. The whole larval period occupies, if there is no hibernation, about ten weeks, and if there is hibernation about five to six months.

Pupa. The so-called pupal period, which may be taken to include the whole time spent within the cocoon, is extremely variable. In summer it may be only about 35 days and in spring about three months, because those that cocoon early in the year do not change into pupa until after about thirty days.

The whole period of development will be at least about four months when the larva does not hibernate, and about eight months when it has to pass through a winter.

SEASONAL APPEARANCE.

There are two distinct, not successive, broods in the year, one which comes out of eggs laid in spring, which may be called the spring brood, and another from eggs laid in late summer and autumn, which may be distinguished as the summer brood.

The spring brood. The beetles of this brood originate from eggs laid in early spring by adults of the previous year's spring brood which have succeeded in hibernating. The egg-laying seems to take place from March to May and the adults emerge towards the end of July and early in August. Breeding takes place almost entirely in roots of charlock (*Sinapis arvensis*). No other host plant has been noted. Though plenty of cultivated crucifers are available at this time they are not attacked. The parents seem to die off by summer. The fresh imagines do not reach sexual maturity till the following spring.

The summer brood. This is the brood which is of direct economic importance. The parent beetles emerge about the beginning of June, feed for a fortnight and then for nearly four weeks hide in the soil. Sexual activity begins about the end of August; the beetles leave their hiding places, feed voraciously, copulate and begin to lay eggs in cultivated crucifers. The adults die off by winter. The eggs generally hatch before winter and the larvae hibernate within the galls in winter. In spring they resume feeding and when full-fed and the soil around has been well moistened by rains leave the galls and pupate in the soil. March and April are the usual periods for pupation. The imagines emerge late in May or early in June.

THE QUESTION OF RACES.

When describing the two broods that appear within one year points were brought out which show that they are racially different; one brood follows the other in time, but in parentage they come from two distinct lines, each line producing one generation in the year. We have, therefore, two distinct races of *C. pleurostigma.*

(i) The spring race, which breeds in spring in charlock, *Sinapis arvensis*, and passes winter in the adult stage.

(ii) The summer race, which breeds in summer and autumn in crops such as turnips and swedes, *Brassica campestris*, and in cabbages and allied plants, varieties of *B. oleracea*, and passes winter in the larval stage.

There are no morphological differences to suggest that these belong to two different species. The chitinous parts of the male genitalia (Text-fig. 12) in individuals of the two races are exactly alike.

PROGRESS OF INFESTATION.

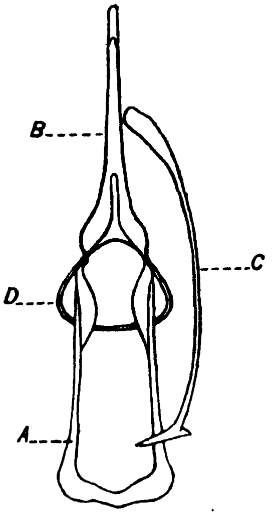
This aspect of the present investigation, being of the greatest moment from the economic standpoint, has been very carefully gone through. Various cultivated crucifers were kept growing throughout the year and wild crucifers were closely watched.

In spring there are only a few ovipositing individuals and since they all belong to the spring race they lay eggs only in charlock, which at this time of the year is in a very flourishing condition. Young brussels-sprouts, cabbages and even turnips are practically left alone at this time.

Those beetles that lay eggs in spring die off before summer, and therefore early in the latter season there are no imagines to lay eggs.

From August to the end of the year eggs are laid exclusively in cultivated crucifers. The large number of adults of the summer race begin to reproduce during the last quarter of August. From now on, specially during September and early October, crops become rapidly and extensively infested and as a result galled. Spring cabbages in the nursery and turnips are the first favourites. When all these have been nearly gone over older crops are attacked. It has been noticed that when turnips and swedes are grown together, turnips are the first to be badly attacked.

During the year 1919, in September, a count was taken of the progress of infestation in a cabbage nursery at Heston. The percentage of plants with galls on the various dates of examination was as follows: 6th, 30 per cent.; 8th, 30 per cent.; 10th, 55 per cent.; 15th, 66 per cent.; 17th, 70 per cent.; 29th, 78 per cent.



Text-fig. 12. Chitinous parts of male genitalia of *C. pleurostigma* (reared from galls on cabbage). Ventral aspect. *A*, body; *B*, femora; *C*, rod or fork; *D*, ring. (× 52.)

In 1920 on the 13th October, every plant on one square yard of ground in a spring cabbage bed was examined. Out of 164 plants 96 had one or more galls on them, *i.e.* 60 per cent. of the crop was affected, which estimate seems from observation to be well the average. At the same time of the year in a turnip plot measuring half a rod every turnip harboured a few larvae.

DISSEMINATION.

The beetles have not been observed in flight, but they do spread out during the ovipositing season from field to field in search of fresh plants.

When young spring cabbages are taken from the nursery and planted over various plots, since they usually have a number of eggs and larvae in them, it is certain that the operation serves to spread the pest. Further, this crop and cultural operations connected therewith afford unhampered facilities for the successful breeding of the weevil. Autumn turnips and swedes are consumed before winter and the fate of the larvae in them is doubtful. But spring cabbages carry the larvae through the winter. They are harvested in spring up to the end of April generally. The stalks are either pulled out as soon as the heads have been cut and spread on pasture lands for cattle to feed on or are left in the ground, which is subsequently ploughed up, allowed to dry and then carted away usually late in April to be burned. But the larvae leave the plants in March and April, and provided the infested stalks remain exposed to rain on the soil large numbers of the larvae in them will emerge and pupate.

NATURAL ENEMIES.

The ordinary garden slug, *Limax maxima*, is, to a small extent, a check on the weevil. Though the slug is a vegetable feeder it eats the grub when it feeds on the galls, and slugs have always been observed to have a special liking for the inner tissues of the galls and their habit is to bore right into the gall. While feeding thus they do not show any dislike for the fat whitish weevil grub. The probability is that they develop a taste for them (Plate V, fig. 5).

The larva of *Helophorus rugosus* Ol. (Hydrophilidae) occurs in the soil in certain fields and has been observed to bore into the gall in preference to other parts of the plant, and also to feed on the weevil grubs. The adult is known as the turnip mud beetle. The larva has often been seen to bore right into the gall, casting away the plant tissues bitten out in the process, and thus making a clear effort to get at the weevil larva inside. These larvae are very shy when exposed to light, but more than once they have been taken with the weevil grubs between their mandibles and when kept in cages with galls they burrow and empty the gall of the occupant and also feed on the inner tissues. When there is a cluster of galls they pass from one into another in search of weevil grubs. It may be interesting to note here Fowler's (3) statement that Perris discovered the larva of one of the species of the sub-genus *Empleurus* Hope (to which *H. rugosus* also belongs) in cabbage stalks devouring the larva of *Halitcidae.*

A Braconid, *Diospilus oleraceus* Hal. has been found as an active parasite on the gall-weevil grubs. The female thrusts her egg into the grub through the gall and the larva feeds inside the host. A parasitised grub lives until it emerges and cocoons. The parasite now emerges from the host and the latter dies. It spins a water-tight silken cocoon inside the host's cocoon, taking care to exclude the dead body of the host. In this cocoon it pupates and the imago bites its way out. The period spent in the cocoon is about a week less than what the host would have in the usual course taken for its metamorphosis. If the host grub is prevented from emerging out of the gall by artificial methods, such as keeping the soil dry, the parasite will after some waiting emerge and cocoon inside the gall, the host dying meanwhile, and the adult will, with its strong mandibles, bite its way out.

The parasite is very active in checking the summer race and large numbers of larvae, especially the earlier ones, are seen to be parasitised, the percentage being about ten for the whole brood. The spring race is not affected by this parasite. The two predators are most active in autumn.

Besides these, various birds peck at the exposed galls and feed on the grubs.

SUMMARY OF OBSERVATIONS.

1. The cause of the smooth round galls on the roots of cultivated crucifers is the presence of the larvae of *Ceuthorrhynchus pleurostigma* Marsh. This pest has a wide distribution over Europe and is common all over the British Isles.

2. The adults feed on the leaves, flowers and young pods of various common cultivated and wild crucifers.

3. The egg is laid in the bark on the root, the larva lives in the gall thrown out by the plant, and the pupa is found in the soil in an earthen cell for constructing which a gummy secretion from the Malpighian tubules is used.

4. There are two distinct races of this insect, a spring race which breeds in *Sinapis arvensis* and not at all in cultivated crucifers, and a summer race which breeds in *Brassica campestris* and *B. oleracea*. The spring race does not injure crops.

5. The summer race begins egg-laying towards the end of August and infests crops. Winter is passed in the larval stage within the galls. Pupation takes place in spring and adults emerge about the beginning of June.

6. If galled plants are kept dry the larvae do not leave the galls but perish inside. If pupation takes place in soil which remains dry several of the pupae die and most of the adults that may develop are unable to break open, and emerge from, the dry pupal cell.

7. The common slug, *Limax maxima*, is fond of the galls, and will often bore right into the galls, feeding on both the plant tissues and the

grubs. Certain birds pick the grubs out of exposed galls. The larva of a Hydrophilid, *Helophorus rugosus*, has similar food habits, and shows a decided preference for the grubs. A Braconid, *Diospilus oleraceus*, is a widespread internal parasite of the weevil grub. When weevil-infested plants with mature grubs are kept dry, though the pest dies within, the parasite, if present, will successfully complete its development, and emerge by biting its way through the dry bark.

BIBLIOGRAPHY.

(1) CONNOLD (1901). *British Vegetable Galls.*
(2) CURTIS (1859). *Farm Insects.*
(3) FOWLER (1891). *Coleoptera of British Isles.*
(4) GAHAN (1900). *Trans. Ent. Soc. London.*
(5) KERNER (1894). *Natural History of Plants.* (Translation by OLIVER.)
(6) KIRBY and SPENCE (1815). *An Introduction to Entomology.* (First Edition.)
(7) —— (1856). *An Introduction to Entomology.* (Seventh Edition.)
(8) KNAB (1915). *Proc. Ent. Soc. Washington.*
(9) KÜSTER (1903). *Pathological Plant Anatomy.* (Translation by FRANCES DORRENCE.)
(10) LEFROY (1915). Xestobium tessellatum *and roof of Westminster Hall*[1].
(11) MARSHALL (1916). *Fauna of British India Curculionidae.*
(12) ORMEROD (1881). *Manual of Injurious Insects.* (First Edition.)
(13) —— (1890). *Manual of Injurious Insects.* (Second Edition.)
(14) STANTON (1912). *British Plant Galls.*
(15) THEOBALD (1907). *Report on Economic Zoology.*
(16) *Journal Board of Agriculture* (England). Vol. XXII. 1915.

[1] Not published. MS. Report to Office of Works.

EXPLANATION OF PLATE V

Fig. 1. Puncture made on young cabbage root for oviposition. (× 7). Fig. 2. Two weevil eggs exposed by removing part of bark from cabbage root. (× 9). Fig. 3. Galls on cabbage root showing exit-holes made by larvae (slightly reduced). Fig. 4. Cocoons made by weevil larvae for pupation. (× 4). Fig. 5. Turnip with galls caused by *C. pleurostigma*. Some of the galls have been bored into by the slug *Limax maxima* (slightly reduced). (Fig. 2 photographed by A. M. Alston.)

(*Received February 3rd,* 1922.)

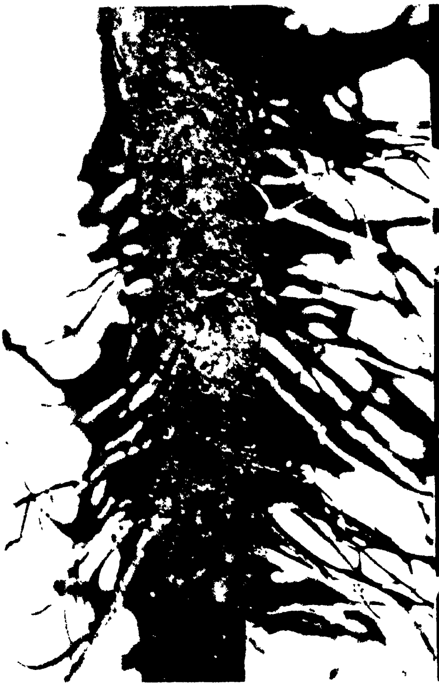


Fig. 1.

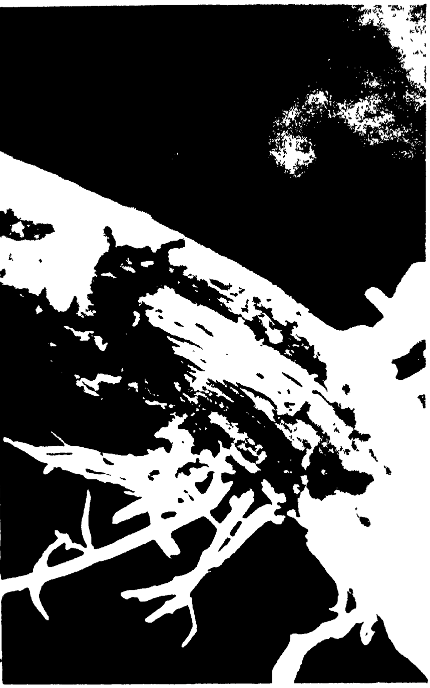


Fig. 2.

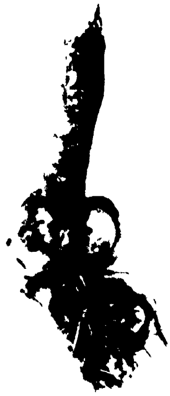
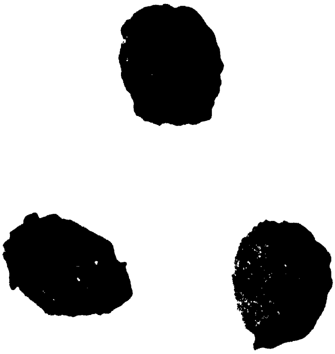


Fig. 3.



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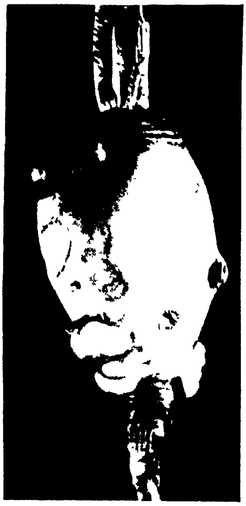

Fig. 5.

THE TURNIP GALL WEEVIL, *CEUTHORRHYNCHUS PLEUROSTIGMA*, MARSH.[1]

PART II. LARVAL ANATOMY

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(With Plates VI and VII and 19 Text-figures.)

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I. MATERIAL AND METHODS.

FULL-GROWN grubs were collected from galls on cabbage roots, fixed in Carnoy's fluid (formula II), and after 24 hours or more were dehydrated by passing into 90 per cent. alcohol for 24 hours, and thence into absolute alcohol for another 24 hours. For de-alcoholising chloroform was found to be unsatisfactory as the larvae floated on the liquid even after 36 hours, showing that the chloroform had not penetrated thoroughly. Xylol was found to be satisfactory in every way.

The imbedding was done by taking these grubs saturated in xylol into a mixture of equal parts of xylol and paraffin of 52° C. kept in liquid condition in the oven. After being in this mixture for four hours they were transferred to melted pure paraffin of 52° C. for four hours and were then imbedded in paraffin of 59° F. Sections were prepared in the usual way and were stained in Delafield's Haematoxylin, and either differentiated by washing in acid 70 per cent. alcohol or counterstained with eosin.

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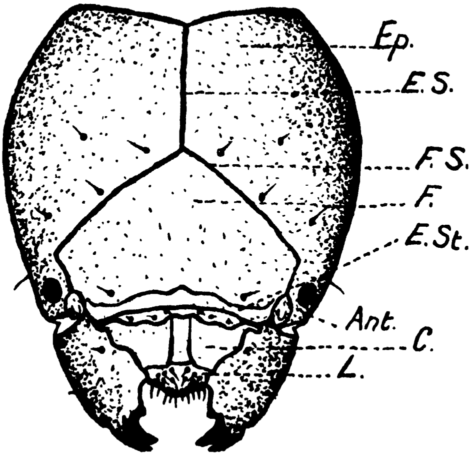
[1] A grant in aid of publication of this communication has been received from the University of London.

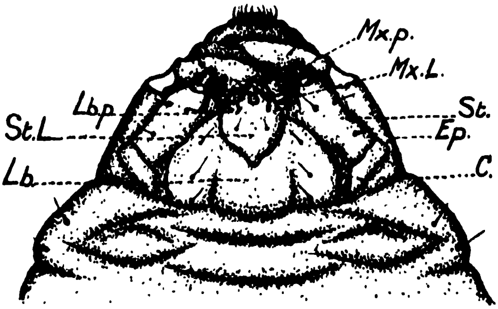
II. EXO-SKELETON.

(1) *The head* (Text-figs. 1 and 2). The head-shield, looked at dorsally, is slightly broader than long; the sides are broadly rounded, the greatest width being near the posterior margin which is angularly emarginate.

From the centre of the emargination along the median line the epicranial suture passes forward dividing the epicranium into two halves.



Text-fig. 1. Dorsal view of head. (× 75.) *Ant.*, antenna; *C.*, clypeus; *Ep.*, epicranium; *E.S.*, epicranial suture; *E.St.*, epistoma; *F.*, frons; *F.S.*, frontal suture; *L.*, labrum.



Text-fig. 2. Ventral view of head and prothorax. (× 65.) *C.*, cardo; *Ep.*, epicranium; *Lb.*, labium; *Lb.p.*, labial palp; *Mx.L.*, lacinia; *Mx.p.*, maxillary palp; *St.*, stipes; *St.L.*, stipes labii.

This suture before it reaches the middle divides into the two frontal sutures running on either side of the frons to the anterior margin of the head. The frons is sub-cordate and margined along the base by the epistoma and obtuse angulate laterally, the angles being nearly as wide as the one at the apex. The base is widely emarginate along epistoma for reception of the clypeus, and emarginate at the basal angles for the antenna.

The epicranium curves round to the underside of the head and stops short at the lateral margins of the fleshy maxillo-labium.

In front of the frons, is the broadly transverse clypeus and anterior to it the distally rounded labrum, the clypeus and labrum together forming a sub-triangular plate overlapping the mandibles.

The labrum is transverse, sub-triangular, nearly twice as broad as it is long, and has its sides sinuate. It bears on the dorsal aspect an irregular circle of 20 spines of which ten are marginal, six sub-basal and four sub-apical. On the ventral aspect there are along the apical margin a number of minute tubercles.

The mandibles are rather stout, triangular and tridentate. The apical and median teeth are sub-equal and sharply pointed, the basal one is short and blunt.

The maxillae are very prominent on the ventral aspect. The cardo is small, the apparent stipes is very large and bears an internal spinose lacinia and an external two-jointed maxillary palp. The labium has a large membranous lobe, consisting of mentum and submentum, contiguous basally with the integument of the prothoracic sternum and laterally with the maxillae. Anterior to this mento-submental lobe and just below the mouth opening is a shield-shaped chitinous area known as the *stipes labii*, bearing on its anterior margin a pair of two-jointed labial palps. On the ventral aspect of the opening of the mouth there is a minute transverse fold, the hypopharynx. The epipharynx has no special outgrowth, being merely a sensitive palatal inner surface of the clypeo-labrum forming the roof of the mouth.

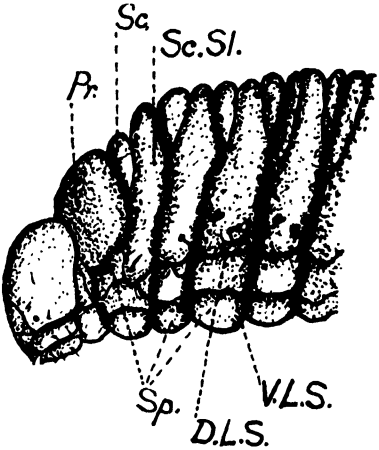
The eyes are extremely rudimentary and are represented by two dark spots on either side of the epicranium close to the base of the mandibles. There is no lens. The antennae also are rudimentary and are found near either basal angle of the frons as small two-jointed processes. The basal segment is very short and flattened, and bears around the base of the small dome-like second segment six very minute spines.

(2) *Spines*. On the head there are a number of spines evidently tactile in function which have a definite arrangement. Their position has been carefully noted. On the basal margin of the frons at each angle close to the indentation for the mandibular condyle, and close to the antenna, there is a single short spine, and posterior to it and closer to the mid region just behind the epistomal suture there is another larger spine. Each lobe of the epicranium bears one spine laterally just behind

the eye and dorsally along the frontal suture a row of three, one close to the epicranial suture, the second more remote but nearer the frontal suture than the other two; a fifth spine occupies the middle position. The clypeus has no spines. The labrum has at its apex a group of 20 hook-like spines whose position has been already described. The mandibles bear three spines, two dorsally and one ventrally, all near the mid-region and close to the outer margin. The maxilla has on its ventral aspect three spines all on the stipes, one close to the cardo and two near the base of the palp. On the labium the stipes labii has one pair occupying a middle position, and on the mento-submental area three pairs, all nearly equidistant, and one behind the other.

(3) *The thorax and abdomen* (Text-fig. 3). The thorax and abdomen bear no appendages and the chitinisation is uniform and smooth except on the dorsum of the prothorax where it is thicker and granulate.

Three thoracic and nine abdominal segments are distinct, and a minute tenth abdominal segment is also to be seen. In freshly moulted specimens two lateral longitudinal folds, the dorso-lateral suture and the ventro-lateral suture, are present dividing each segment into tergal, pleural and sternal areas. The prothoracic tergum is undivided and large. The mesonotum and metanotum have a small scutum and a large scuto-scutellum. The abdominal tergites have their segmentation usually indistinct owing to the swollen condition in which the larva generally is.



Text-fig. 3. Head and forepart of body. (× 25.) *D.L.S.*, dorso-lateral suture; *Pr.*, prothorax; *Sc.*, scutum; *Sc.Sl.*, scuto-scutellum; *Sp.*, spiracle; *V.L.S.*, ventro-lateral suture.

At the lateral extremities of all the abdominal tergites just above the dorso-lateral suture, on what may be termed the epipleural lobe, there is a small tubercle bearing a pair of spines of which the anterior one is slightly smaller. The meso- and metathorax do not have these tubercles and have in that region a single spine in place of the pair on the abdomen. The prothoracic epipleural lobes have neither the tubercle nor the spine. A few spines are distributed over other parts of the body.

There are nine pairs of spiracles one on the mesothorax and one on each of the first eight abdominal segments. The abdominal ones are placed on the epipleural lobes, a little above and anterior to the spine-bearing lateral tubercle. The mesothoracic one is also on the epipleural lobe, above and anterior to the lateral spine already mentioned. But owing to the expansion and thickening of the pronotum, the part of the epipleural lobe which carries this spiracle appears to lie below the prothoracic shield and close to the head, and it has therefore often led to the wrong statement that the only pair of thoracic spiracles present belongs to the prothorax.

III. THE INTEGUMENT.

(Text-fig. 4.)

The integument is made up of an outer chitinous cuticula and an underlying cellular layer. Under high magnification the cuticula appears to be in two strata, an outer homogeneous and dense primary cuticula and a thicker lamellate lower layer, the secondary cuticula. The cellular region shows a thick undifferentiated protoplasmic layer with scattered nuclei—the hypodermis—and below it a uniform hyaline basement membrane.

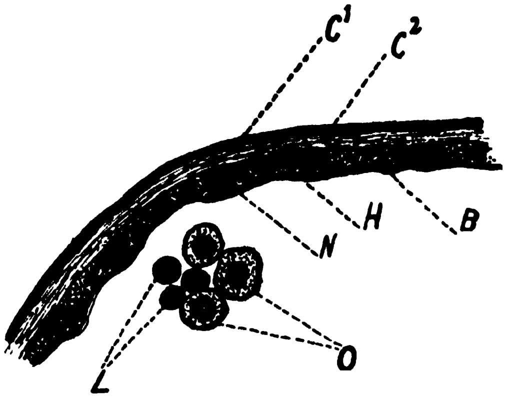
IV. ENDO-SKELETON.

The tentorium (Text-fig. 5). Within the head is a chitinous supporting structure, the tentorium. It consists of a middle transverse plate attached by means of two lateral processes to the ventral margin of the epicranium. Two narrow long anterior and two short posterior arms are also given off. The tentorium lies below the oesophagus. The anterior branches are reflected towards the clypeus and lie on either side of the pharynx. Besides bracing the cranium and holding the cephalic ganglia and oesophagus in place, the tentorium also affords attachment to certain muscles in the head. The central plate is hollow and the whole tentorium is enveloped by a thin epithelial layer resembling the hypodermis.

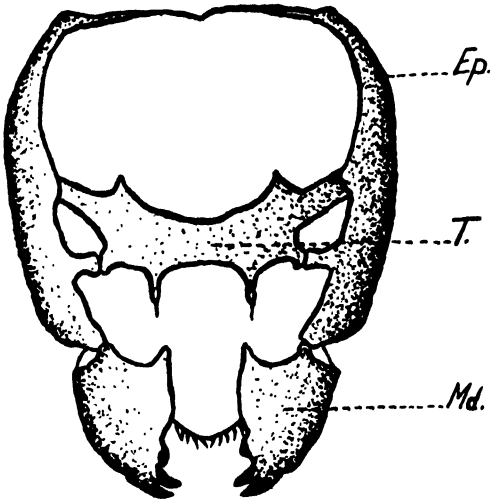
V. THE NERVOUS SYSTEM.

(Text-fig. 6.)

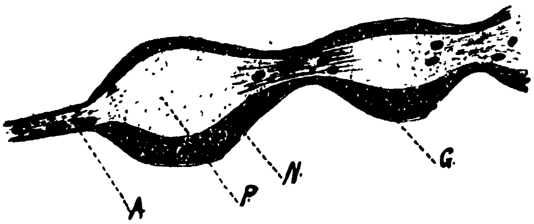
The central nervous system consists of the bilobed supraoesophageal ganglion situated at the back of the head and slightly protruding into the prothorax, connected by oesophageal commissures to the sub-oesophageal ganglion which lies ventrally just above the cuticular fold between the labium and the prosternum, and a double ventral nerve cord with three thoracic and seven abdominal ganglia, the last ganglion being rather elongate and composed of the fusion of a number of ganglia. In transverse section a ganglion exhibits (1) an internal clear medullary portion (Punktsubstanz), and (2) a dense outer layer of ganglion cells, and (3) a thin enveloping membrane, the neurilemma.



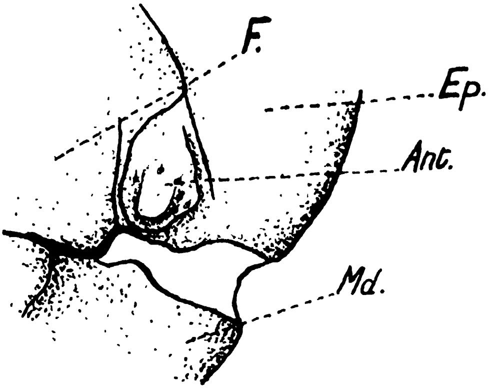
Text-fig. 4. Integument, leucocytes and oenocytes. (× 700.) *B.*, basement membrane; C^1, primary cuticula; C^2, secondary cuticula; *H.*, hypodermis; *L.*, leucocyte; *N.*, nucleus; *O.*, oenocyte.



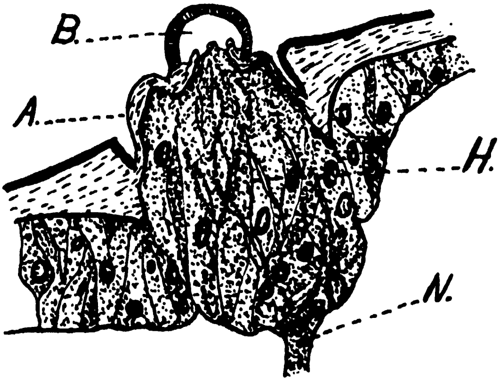
Text-fig. 5. Ventral view of cranium with tentorium in place. (× 80.) *Ep.*, epicranium; *Md.*, mandible; *T.*, tentorium.



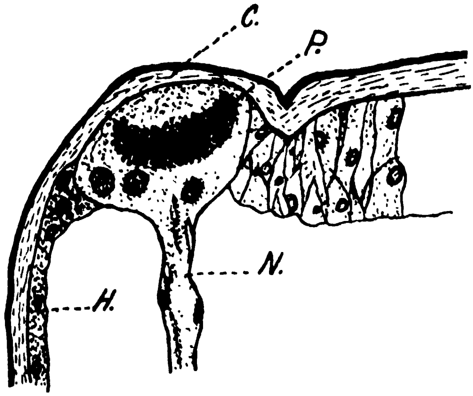
Text-fig. 6. Longitudinal section through the sub-oesophageal ganglion and part of the ventral nerve-cord. (× 180.) *A.*, axis cylinder; *G.*, layer of ganglion cells; *N.*, neurilemma; *P.*, "punktsubstanz."



Text-fig. 7. Antenna. (× 200.) *Ant.*, antenna; *Ep.*, epicranium; *F.*, frons; *Md.*, mandible.



Text-fig. 8. Median section through antenna. (× 600.) *A.*, basal joint of antenna; *B.*, second joint of antenna; *H.*, hypodermal cells; *N.*, nerve connection.



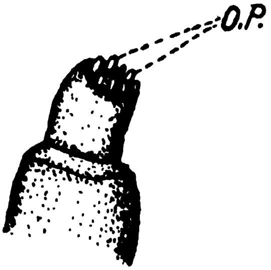
Text-fig. 9. Median section through eye-spot. (× 540.) *C.*, chitin; *H.*, hypodermis; *N.*, nerve fibre; *P.*, pigment granules.

A nerve-cord consists of a fibrous axis-cylinder with scattered nuclei and the enveloping neurilemma.

The sense-organs noted are the antennae, the eyes, the sensory spines and the olfactory pegs.

The antenna (Text-figs. 7 and 8) has already been described. In a longitudinal section the basal joint appears to have its chitin much thinner than that on the surrounding area. It bulges out only slightly from the head. The second joint is rather dome-shaped and has fine radial striae in the chitin. The eyes (Text-fig. 9) are very little developed and probably are of use only to perceive light. There is no lens. There is only a crowding together of hypodermal cells and a thick concretion of dark pigment granules within them. The cells seem to have their nuclei slightly larger than those in the neighbouring hypodermis and a nerve connection to the eye-spot can also be traced. These eye-spots bear a striking resemblance to the simple optic pits found in certain molluscs.

The distribution of the chief sensory spines on the head has been already recorded. Besides these there are several other sensory spines on the body. The olfactory pegs (Text-fig. 10) are certain small conical processes on the tips of the maxillary and labial palps. The name has been recently suggested by McIndoo, and is more convenient than the term sensillum styloconicum used by Folsom. The pegs are borne on a very thin chitinous disc. There are eight of them on the maxillary palp and about seven on the labial palp.



Text-fig. 10. Maxillary palp. (×500.) *O.P.*, olfactory peg.

VI. THE DIGESTIVE SYSTEM.

The alimentary tract exhibits the three primary regions, the stomodoeum or fore-gut, the mesenteron or mid-gut (stomach) and the proctodoeum or hind-gut. The whole tract measures in a full-grown larva nearly 11 mm. The stomodoeum joins the mesenteron in the second thoracic segment.

There are a pair of short salivary glands. The mesenteron has no gastric coeca. Six Malpighian tubules, of which two are shorter than the rest, are also found.

The alimentary canal has its wall made of four different layers; there is an innermost intima of uniform structure, then a single layer of cellular epithelium, next to it a thin basement membrane and outside all a muscular layer.

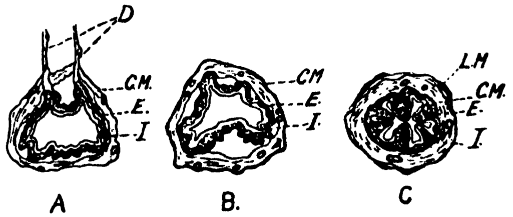
(i) *The stomodoeum.*

(*a*) *General.* The mouth opens between the labrum and the maxillolabium. Directly behind the mouth the intestine swells a little to form a pharynx. Behind the pharynx is a narrow short oesophagus leading into the next region which has its inner wall thrown into six longitudinal folds. It may be called the proventriculus, since though it is not swollen it corresponds in structure and function to the so-called area in other insects. The stomodoeum now loses the internal ridges, swells a little, forms the oesophageal valve, and then meets the mesenteron. The transformation from stomodoeum to mesenteron is abrupt.

(*b*) The intima is a homogeneous and chitinous layer investing the entire fore-gut, anteriorly contiguous with the chitinous cuticula covering the labium and epipharynx. It forms a sort of loose cover with irregular folds and protects the epithelium below. The stomodoeal epithelium is thin and compact with the nuclei distinct. The basement membrane is very thin and nucleated, and closely envelopes the epithelium. The muscular layer is well developed and is composed almost entirely of circular muscles, but a few longitudinal muscles are also present on the outside of the layer of circular muscles. These muscles evidently, by peristaltic action, push the food from the fore-gut into the stomach.

The Pharynx, Oesophagus, Proventriculus and the Oesophageal Valve.

The Pharynx (Text-fig. 11 A) in transverse section is somewhat triangular in shape with a broad basal area. Along the dorsal aspect the epithelium is folded into a ridge jutting into the lumen. The circular muscles form a thick covering. Dorsally, certain paired divaricatory muscles running down from the epicranium pierce through the circular muscle layer, and become attached to the epithelium.

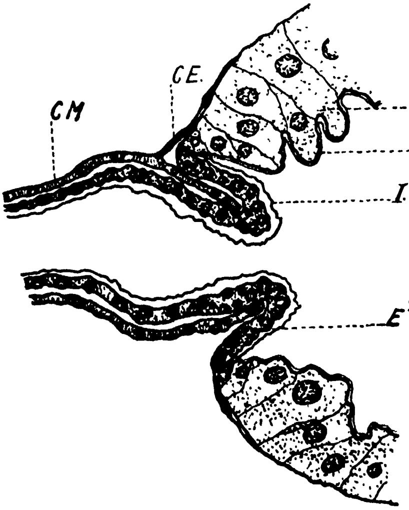


Text-fig. 11. Transverse section through *A*, pharynx; *B*., oesophagus and *C*., proventriculus. (× 150.) *C.M.*, circular muscles; *D.*, pharyngeal divaricators; *E.*, stomodoeal epithelium; *I.*, intima; *L.M.*, longitudinal muscles.

The Oesophagus (Text-fig. 11 B). In the oesophagus the epithelium is thrown into irregular waves.

The Proventriculus (Text-fig. 11 c) is slightly narrower than the other region of the stomodoeum and almost circular in section. The epithelium has six definite ridges which project inward so far as nearly to meet. The circular muscles are pronounced and a few longitudinal muscles are also present. The chitinous intima does not exhibit any development of teeth, but the whole structure of this region indicates that its function is to squeeze and strain the food.

The Oesophageal Valve (Text-fig. 12). Just before the stomodoeum joins the mesenteron it runs into the latter and then reflects on itself, thereby forming a tubular lip which acts as a valve preventing the backward passage of food from the mesenteron. To receive this valve the mesenteron at its anterior end forms a somewhat pear-shaped cavity known as the cardiac chamber. The stomodoeum itself swells a little before the valve to serve as a receiving chamber for food that has to be passed through the valve. The muscular layer is not reflected with the other layers, but ends at the neck where the backward folding starts, but a few longitudinal muscle strands branch off from the stomodoeal layer and run over the cardiac chamber. These may be termed the cardiac extensors. The epithelium shows no change but the cells are more closely arranged in the area of the fold. The chitinous intima retains the appearance of a loose cover. The transition to the mesenteron is clearly marked



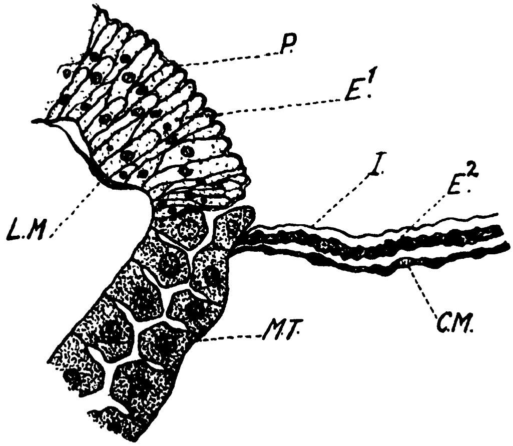
Text-fig. 12. Oesophageal valve. (× 220.) *C.E.*, cardiac extensor; *C.M.*, circular muscles; E^1, epithelium of mesenteron; E^2, epithelium of stomodoeum; *I.*, intima; *P.*, peritrophic membrane.

by the large columnar epithelial cells of the latter and its closely applied peritrophic membrane.

The action of the valve is probably regulated as follows: masticated and strained food reaches the receiving chamber. The cardiac extensors contract pulling out the sides of the cardiac chamber, thereby increasing its capacity and tending to produce a partial vacuum. This process also allows the tubular lip of the valve to extend out. The peristalsis of the muscles of the stomodoeum drives the food through the valve into the mesenteron. The return of food is prevented by the valve closing automatically when pressure is applied from within the mesenteron.

(ii) *The Mesenteron.* (Text-fig. 13.)

In this region only a few muscle strands are seen, and probably all are circular. The epithelium is made up of large columnar cells. The intima takes the form of a very thin peritrophic membrane closely applied to the epithelium. The Malpighian tubules open into the intestine at the junction of the mesenteron and proctodoeum.

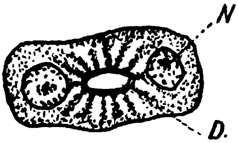


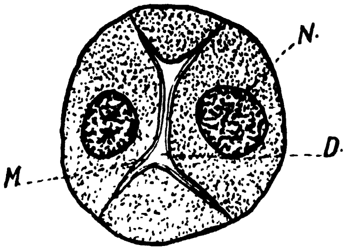
Text-fig. 13. Origin of a Malpighian tubule. (× 150.) *C.M.*, circular muscles; *E*¹, epithelium of mesenteron; *E*², epithelium of proctodoeum; *I.*, intima; *L.M.*, longitudinal muscle; *M.T.*, Malpighian tubule.

(iii) *The Proctodoeum.* (Text-fig. 13.)

In this part the alimentary canal is very narrow and much convoluted. The intima is chitinous and loose and near the oval opening thrown into irregular folds. The epithelial cells are small and closely arranged as in the stomodoeum. The muscular layer is very thick and made up mostly of circular muscles.

The Salivary Glands (Text-fig. 14 and Plate VI, fig. 1). There are two very minute tubular salivary glands each about 45 mm. long, lying on the ventral aspect below the stomodoeum and the tentorial plate. They open separately on the labium just anterior to the hypopharynx. The epithelial cells of the glands are very large with large nuclei. There is a central canal running the whole length of the tube.





Text-fig. 14. Transverse section through salivary gland. (× approx. 600.)

Text-fig. 15. Transverse section through Malpighian tubule. (× 300.) *D.*, duct; *M.*, investing membrane; *N.*, nucleus.

The Malpighian Tubules (Text-fig. 15 and Plate VI, fig. 2). There are six Malpighian tubules, of which two are small and probably do not function. The former in a full-grown larva are nearly 8 mm. long. Each is made up of large polygonal cells. For a length of about 2 mm. at their basal ends these tubules are very narrow, probably this portion serves as a duct. The two small tubules are each about 3 mm. long and along their whole length are as narrow as the ducts of the others. In transverse section a tubule shows a ring of four large cells, protruding into the lumen, and leaving between them a narrow continuous space. A membrane invests the inner free surfaces of the cells.

VII. THE CIRCULATORY SYSTEM.

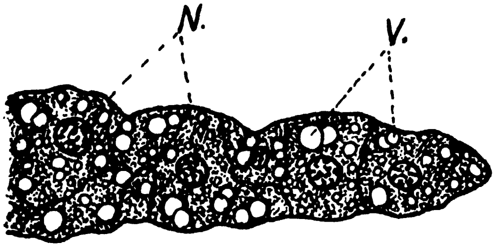
This system seems to consist merely of a simple short tube dorsal to the alimentary tract and extending from the mesothorax to the supra-oesophageal ganglion; just behind the ganglion this tube appears triangular in transverse section with the base above the stomodoeum, but posteriorly it tapers and becomes flattened from side to side and no trace of it is seen in the metathorax. How exactly the dorsal vessel ends anteriorly it has been difficult to note. In structure it has a wall of fine muscle fibres.

VIII. THE BLOOD TISSUE.

(Text-fig. 4.)

Here and there in the plasma small rounded nucleated cells are seen singly or in groups of a few, these are the leucocytes, otherwise known as phagocytes. Besides these certain large cells are also seen free; these are the Oenocytes.

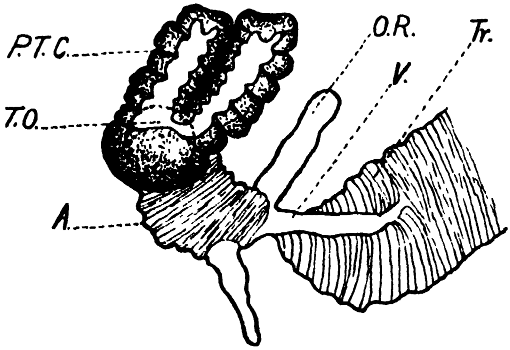
Intimately connected with the blood is the fat body (Text-fig. 16) which occupies a large portion of the body cavity. It has no definite form and more or less surrounds the various internal organs. The individual fat cells are not clearly defined but their nuclei and numerous vacuoles filled with globules of fat are quite clear.



Text-fig. 16. Fat cells. (× 430.) *N.*, nucleus; *V.*, vacuoles.

IX. THE RESPIRATORY SYSTEM.

There are two lateral tracheal trunks with numerous ramifications. It has been noted that there are nine pairs of spiracles. The abdominal ones are all nearly equal, but the last two are slightly larger. The mesothoracic one is distinctly larger than the rest.



Text-fig. 17. Spiracle. (× 530.) *A.*, atrium; *O.R.*, occlusor rod; *P.T.C.*, peritremal tracheal chamber; *T.O.*, tracheal orifice; *Tr.*, spiracular branch of lateral tracheal trunk; *V.*, vestibule.

The trachea is constructed on the usual lines and has a chitinous lining or intima strengthened on the inner face by thread-like ridges, the taenidia which course in a spiral manner, and an outer layer of tracheal hypodermis.

The spiracles (Text-fig. 17) do not communicate directly with the outside; instead they open into a thin walled U-shaped chamber in the chitin. This chamber has its two arms strengthened by minute transverse internal ridges. To this chamber the term peritremal tracheal chamber may be applied. A narrow slit runs along the roof of the chamber over both arms and affords communication with the outside air. The lips that guard this slit seem to have the power to come together or open out and thus regulate the vent according to the humidity of the surrounding atmosphere.

A circular opening, the tracheal orifice, leads from the peritremal tracheal chamber into a drum-shaped atrium. The atrium at its inner extremity gives out two short hollow chitinous peg-like processes, the occlusor rods, and then leads into a narrow vestibule which runs on to a side of the spiracular branch of the lateral trunk communicating with it by means of a lateral opening.

The two peg-like processes and the narrow vestibule which is only partially chitinised form the closing or occluding apparatus of the stigmata.

The occlusor rods are connected at their basal ends by very minute occlusor muscles (Plate VI, fig. 3). By their contraction these can bring nearer the two rods and make their bases press against the trachea, and thus regulate or close the opening from the atrium into the vestibule. These muscles consist only of a few cells and are so small that they are distinct only in some of the sections.

In a larva under natural conditions the vent in the peritremal tracheal chamber appears closed and respiration seems to take place directly through the cuticle, below which numerous tracheal branches are found. This adaptation is quite to be expected as the larva is usually living under very moist conditions and while within the gall the method of respiration is certainly akin to that under aquatic conditions. But when the larva is kept in dry open surroundings as in dry soil for a few days and then examined, the vents appear open.

X. THE MUSCULAR SYSTEM.

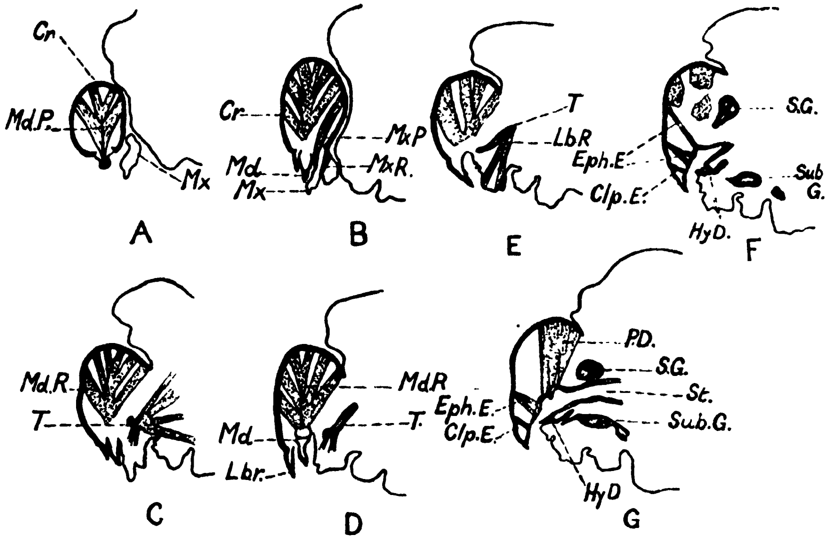
The muscles present a striated appearance and each muscle is a bundle of long fibres with nuclei in different parts and enveloped in a membrane, the sarcolemma.

Besides the muscles connected with the digestive canal and the heart there are three other systems within the body, the cranial muscles controlling the oral appendages, the cephalic retractors and a network of subcutaneous muscles.

The cranial muscles (Text-fig. 18 *A*, *B*, *C*, *D*, *E*, *F* and *G*). The muscles in the head are mainly those controlling the mouth appendages.

In the cavity of the epicranium there is on either side, first the mandibular protractors, and then the mandibular retractors, both very strong sets of muscles attached to the mandible by means of chitinous ligaments from which a thin chitinous plate protrudes into each muscle-set, helping to make the attachment very strong. Ventral to, and almost between these, are the maxillary elevators and below them the maxillary depressors.

In the mid-dorsal region of the cranial cavity, between the mandibular retractors and in front of the supra-oesophageal ganglion are first a few thin elevators of the clypeus and then a few elevators of the epipharynx and behind them the paired fan-shaped pharyngeal divaricators.

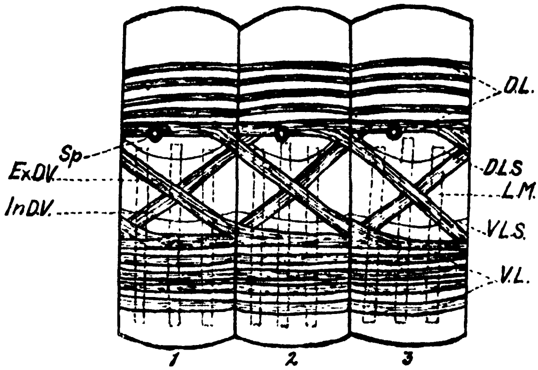


Text-fig. 18. *A*, *B*, *C*, *D*, *E*, *F* and *G*. Sagittal sections in series through head and prothorax; *A*., outermost and *G*. nearly median longitudinal section to show arrangement of cranial muscles. (× 28.) *Clp.E.*, elevator of clypeus; *Cr.*, cranium; *Eph.E.*, elevators of epipharynx; *Hy.D.*, depressors of hypopharynx; *Lb.*, labium; *Lbr.*, labrum; *Lb.R.*, retractors of labium; *Md.*, mandible; *Md.P.*, mandibular protractors; *Md.R.*, mandibular retractors; *Mx.*, maxilla; *Mx.P.*, maxillary elevators; *Mx.R.*, maxillary depressors; *P.D.*, pharyngeal divaricators; *S.G.*, supra-oesophageal ganglion; *St.*, stomodoeum; *Sub.G.*, sub-oesophageal ganglion; *T.*, tentorium.

On either side of the tentorium ventrally from its posterior margin are given out two muscles, one anterior and one posterior, both being apparently retractors of the labium. From the middle of the anterior margin of the tentorium are given out two very short muscles, one above the other, the median depressors of the hypopharynx, their function being in conjunction with the clypeal and epipharyngeal elevators to open out the mouth aperture.

The cephalic retractors. These are powerful paired muscles in a mid-dorsal position in the prothorax attached anteriorly to the hind margin of the epicranium and posteriorly to the hind region of the prothorax, they draw the head into the prothorax and also elevate it to a small extent.

The subcutaneous muscles (Text-fig. 19). These muscles form a tangled network below the cuticle. The chief muscle-strands seem to be in a regular series repeated in every body segment. They are in two distinct lateral sets, there being practically no muscle strand crossing over from one side to the other, either in the mid-dorsal or mid-ventral line.



Text-fig. 19. Diagrammatic representation of the arrangement of the subcutaneous muscles in the body-segments. 1, 2, 3—three consecutive body-segments, 1 being nearest the head. *D.L.*, dorso-lateral longitudinal muscles; *D.L.S.*, dorso-lateral suture; *Ex.D.V.*, external dorso-ventral oblique muscles; *In.D.V*, internal dorso-ventral oblique muscles; *L.M.*, lateral muscles; *Sp.*, spiracle; *V.L.*, ventro-lateral longitudinal muscles; *V.L.S.*, ventro-lateral suture.

Just below the hypodermis there are a few slender transverse muscles —the *lateral muscles.* These originate irregularly from points roughly along the dorsal longitudinal fold and are inserted on the ventral wall close to the mid-ventral line. There are three of these on every side in each segment, but they are not all clear in the thorax. The ninth and tenth abdominal segments are too minute to show either these or other muscles. These lateral muscles are of use in drawing closer the dorsal and ventral regions and generally in slight wriggling movements.

Below these are the *external dorso-lateral oblique muscles* and next to them the *internal dorso-lateral oblique muscles.* The external dorso-lateral oblique muscles originate from a point at the junction of two segments, close to the dorso-lateral suture which marks the junction of the tergal and pleural areas and are inserted at a point in a line with the ventro-lateral suture in the next posterior junction of two segments.

The *internal dorso-lateral oblique muscles* have their points of origin and insertion exactly the reverse of what they are with the externals. So that in the pleural region of every segment these muscles cross one another and form an X. At their points of attachment these muscles do not end abruptly but also send branches, some of which merge into the longitudinals, and others which go further on to the tergal or sternal cuticle. Those to the tergite are fairly well marked, but the others are often indistinct.

There are two sets of longitudinals on either side. These are continuous from the prothorax to the tip of the abdomen. First, there are the *dorso-lateral longitudinal muscles*. They are in very thin strands and are attached to the chitin along the inter-segmental junctions. Then there are the *ventro-lateral longitudinal muscles* forming a very thick strong system. They are also attached to the cuticula along the junctions of the body segments.

In the prothorax the internal dorso-lateral oblique muscles and the ventro-lateral longitudinal muscles originate in the way described, but the point is also one where the ventral margin of the epicranium meets the anterior lateral arms of the tentorium and forms a hard chitinous knob. These muscles are therefore presumably able to retract the head to some extent.

In the terminal abdominal segments certain minor muscle fibres, which go to the tergum from the points of attachment in the dorso-lateral suture, are very well developed.

When the general arrangement of the muscular system has been made out the mechanism of locomotion suggests itself for elucidation. It has been pointed out in the first part of this paper that locomotion in this larva was brought about not entirely and directly by the muscles but indirectly and mainly by muscular contractions in one region of the body increasing the fluid pressure within and transmitting this pressure through the body-fluid to, and thereby causing movements in, other parts of the body. That the maintenance of the turgidity of the body brought about by the general muscular contraction of the body-wall raising the pressure within the body-cavity above that of the atmosphere is an essential factor in effecting many of the so-called muscular movements in all soft-bodied larvae has been suggested by Prof. H. M. Lefroy. The structure of the larva of *C. pleurostigma*, together with observations on the body movements, provides clear proofs to show that such is the case. A larva when killed by any method loses its turgidity and becomes flaccid. The arrangement of the subcutaneous muscles indicates that their function is to contract the body segments and thus bring about the contraction of a part or the whole of the body. The mouth and anal openings are normally closed due to the pressure of the surrounding body-fluid. The presence of the elevators of the epipharynx, the depressors of the hypopharynx and the pharyngeal divaricators and the thick layer of circular muscles or the coiled proctodoeum, for getting rid of the excreta by peristaltic action, indicate the need to counteract this pressure when necessary.

XI. THE IMAGINAL BUDS.

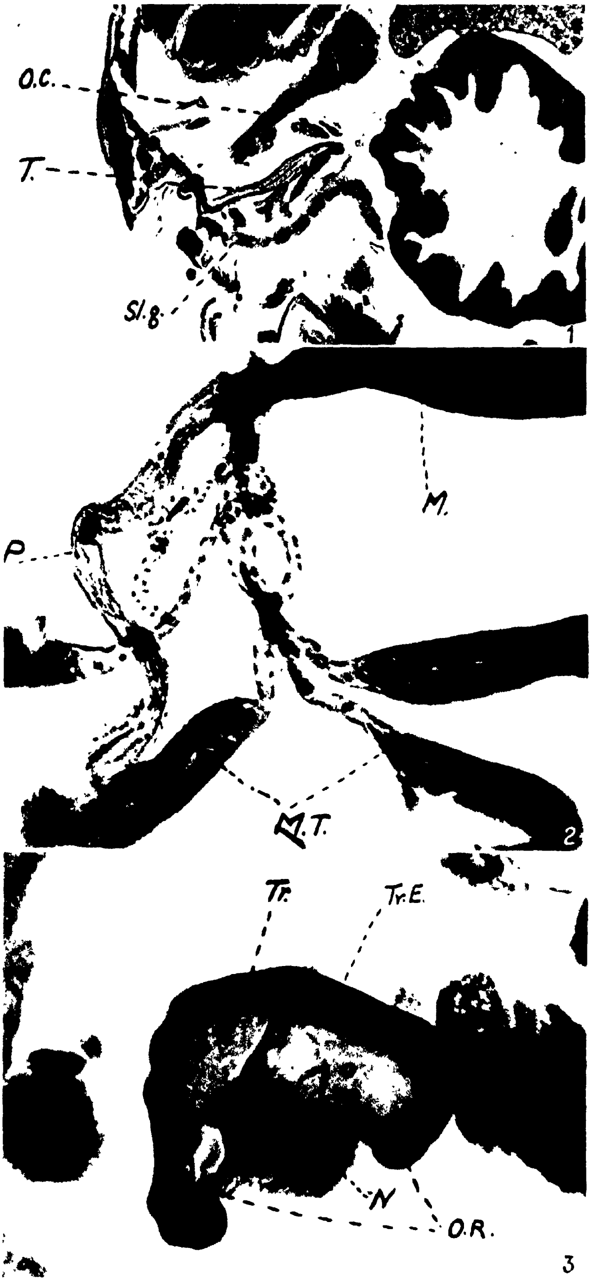
(Plate VII.)

The larvae of holometabolous insects are said to possess certain masses of cells arising from the hypodermis, termed imaginal discs, buds or folds which are the formative centres during metamorphosis of various adult organs.

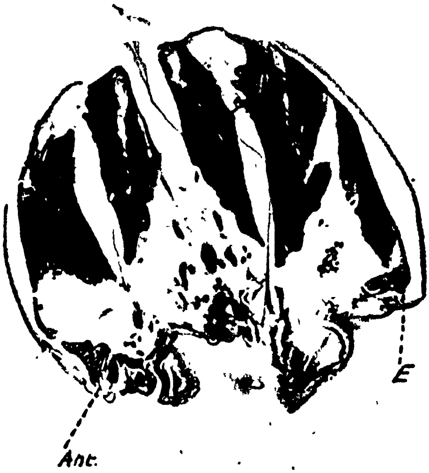
In the full-grown larva of *C. pleurostigma* several such buds are present. Each bud is clearly an evagination of the hypodermis within a cup-shaped cavity formed by the invagination of the hypodermis. There are five pairs of buds distinct, one ventral pair is found in each of the thoracic segments, forming eventually the imaginal legs and one dorsal pair in both the mesothorax and metathorax which grow into the wings. No antennal buds are present and it seems probable that the larval antenna develops into the adult antenna.

XII. BIBLIOGRAPHY.

Berlese, A. (1909). *Gli Insetti.*
Folsom, J. W. (1909). *Entomology.*
Gunther (1912). *Zeit. für Wiss. Zool.* c.
Hirschler (1909). *Ibid.* xcii.
Korschellt, E. and Heider, K. (1899). *Text-book of the Embryology of Invertebrates.*
Krancher, O. (1881). *Zeit. für Wiss. Zool.* xxxv.
Macbride, E. W. (1914). *Text-book of Embryology.*
McIndoo, N. E. (1918). *Journal of Morphology*, xxxi.
Packard, A. S. (1909). *A Text-book of Entomology.*
Perris, E. (1877). *Ann. Soc. Linn. Lyon.* xxiii.
Poyarkoff, E. (1910). *Arch. d'anatomie microscopique* (Paris), xii.
Wheeler, W. M. (1899). *Journal of Morphology*, iii.



ISAAC.—II. The Turnip Gall Weevil. (pp. 171–189.)



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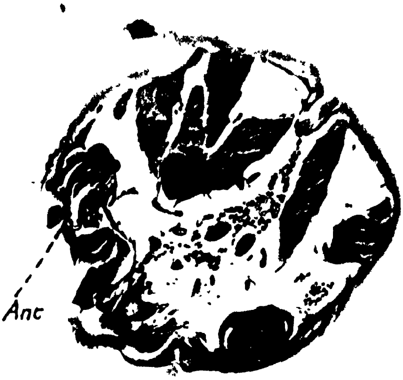

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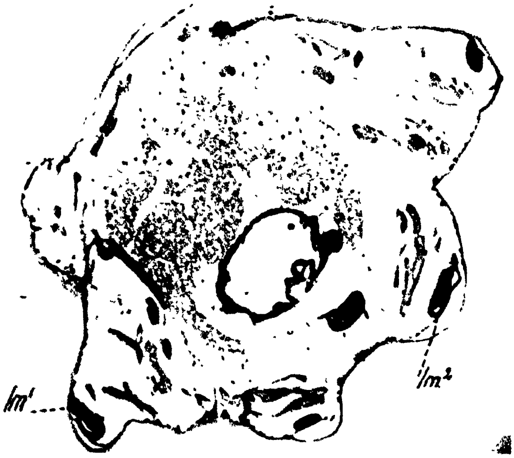

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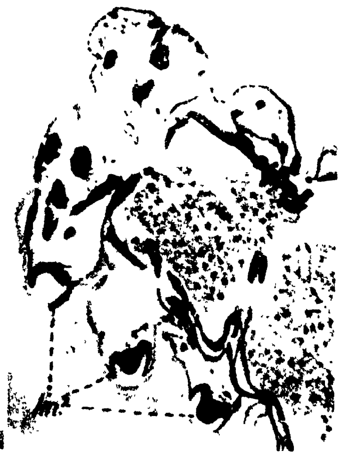

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ISAAC.—II. THE TURNIP GALL WEEVIL. (pp 171–189.)

EXPLANATION OF PLATES VI AND VII

PLATE VI.

Fig. 1. Sagittal section through head and thorax of larva. (× 100.) *O.c.*, oesophageal commissure; *Sl.g.*, salivary gland; *T.*, tentorium.

Fig. 2. The origin of the Malpighian tubules. (× 30.) *M.*, mesenteron; *M.T.*, Malpighian tubule; *P.*, proctodoeum.

Fig. 3. Section through trachea showing occlusor rods and occlusor muscle. (× 800.) *N.*, nucleus of occlusor muscle; *O.R.*, occlusor rod; *Tr.*, trachea; *Tr.E.*, tracheal epithelium.

PLATE VII.

Fig. 1. Transverse section through the head of a larva. (× 90.) *Ant.*, antenna; *E.*, eye.

Fig. 2. Transverse section through nearly the same region as in Fig. 1, in a larva about to pupate. (× 100.)

Fig. 3. Transverse section through the mesothorax of a larva. (× 70.) Im^1, imaginal bud of wing; Im^2, imaginal bud of leg.

Fig. 4. Sagittal section through the thorax of a larva showing the imaginal buds of the legs on one side. (× 75.)

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THE TURNIP GALL WEEVIL, *CEUTHORRHYNCHUS PLEUROSTIGMA*, MARSH.[1]

PART III. CONTROL

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I. INTRODUCTION.

The control experiments included the use of soil fumigants to kill the beetles or deter them from ovipositing, dipping cabbage seedlings in hot water to kill the grubs that may be present in the roots, and the proper disposal of old stalks. As the beetles feed on the inner tissues of leaves and flowers and owing to the nature of the crops to be protected, the use of stomach poisons was barred and for obvious reasons no method that would have entailed much expense was attempted.

II. SOIL FUMIGANTS.

For the trial of soil fumigants six plots of spring cabbages, each one rod in area, were selected. These had been sown about the middle of July, 1920. The application of the fumigants was made between the 11th and 14th of September, when the plants had reached a stage likely to be infested. The substances tried were "tar product," naphthalene, dichlorbenzene, active creosote and chlorcresol. One plot was kept for control.

1. "Tar product." This is a powder consisting of ground neutral residue and chalk and 1 per cent. of neutral tar oil. The powder was mixed with nine times its weight of chalk-powder and 5 lbs. of the mixture was spread on one plot.

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[1] A grant in aid of publication of this communication has been received from the University of London.

2. Naphthalene. Ordinary naphthalene was used with chalk in the same strength and the same amount as the "tar product."

3. Dichlorbenzene. This is a pleasant smelling crystalline powder which evaporates very slowly. This also was applied to the soil in the same manner and strength as the two previous substances.

4. Active creosote. This was mixed with chalk-powder at the rate of 4½ fl. ozs. to 4½ lbs. and spread over the plot.

5. Chlorortho-cresol. This was used in the liquid state with chalk-powder in the same strength and amount as the active creosote.

On October 18th the plots were examined for results of the treatment. From each plot 200 plants were taken out at random and a count made of the number of galled ones. The result was: "tar product," 139; naphthalene, 129; dichlorbenzene, 124; active creosote, 118; chlorcresol, 112; and in the untreated control plot, 131. This indicates that chlorcresol gave the best results, but compared with the control plot, even this did not protect the plants to any satisfactory extent and the entire experiment shows that the use of repellents on a field scale is not likely to be efficacious.

III. HOT WATER TREATMENT OF SEEDLINGS.

Provided it killed the larvae but did not injure the plant, the immersion of the roots of infested cabbages at the time of transplanting in hot water for a fixed time appeared to provide a simple and inexpensive method for the partial control of the weevil. To get a preliminary idea of the effect of such treatment a number of plants were dipped for a fixed time in water kept at a uniform temperature. After five minutes in water at 107·6° F. the larvae were alive, so were they after 10 minutes immersion, but after 15 minutes in the bath they appeared dead and when examined a few hours after the treatment had revived. At 104·9° F. after 30 minutes the larvae were not dead. At 118·4° F. for 5 minutes the larvae appeared dead but were found to have revived the next day. It was therefore decided to try a long immersion at 107·6° F. and a few bundles of plants were treated for 25, 30, and 35 minutes respectively. After 16 hours from the treatment the larvae in the first set resumed activity, but those in the others were found quite dead. Seedlings from the last two bundles were planted out but all wilted away.

It was next attempted to try a higher temperature for a short time. Immersion at 122° F. for 3 and 2 minutes killed the larvae, but plants so treated failed to grow. It is, therefore, to be concluded that the hot water treatment is a failure.

IV. DISPOSAL OF OLD STALKS.

The next point attended to was the disposal of old stalks in ways that would (1) help the Braconid parasite, *Diospilus oleraceus*, to breed out, or (2) prevent the grubs in the galls from reaching maturity. It has been stated that if galled stems are pulled out and allowed to dry under shelter the grubs perish within, also that if infested plants are kept in soil with only a little moisture and protected from rain, the parasite may cocoon in the gall and emerge while the weevil grubs would remain in the galls and die ultimately. To store old stalks under these conditions is an impossibility and therefore experiments along that line had to be given up.

It was found that if old stalks with larvae in them are stacked up in very large heaps the multiplication of the pest may be checked. Such stacking has to be done as early in spring as possible. In a large heap stacked loose the stalks dry quickly, the top ones to a large extent prevent rainwater from wetting those below from which therefore the grubs will not emerge and they themselves being well exposed dry quickly and are also visited by various birds which pick out the grubs.

V. RECOMMENDATIONS.

Taschenberg (5) recommended the rooting out and burning of infested stalk where possible before the larvae leave the galls. He did not think the application of soot or lime or any other such material of any effect in controlling the weevil attack. Miss E. A. Ormerod (3) suggested the burning of old infested stalks, and not to bury them as this will not prevent the grubs from turning into imagines, or throwing them into farmyards where the grubs will be choked in wet manure or shooting them into field-ponds away from houses. She also recommended in the case of seedlings the rejection of galled ones or the scraping off of the galls, as a preventive the application of gas lime or soot or fresh earth to the soil. Ritzema Bos (4) indicated the rooting out and burning of galled plants as the only possible check. Prof. Theobald (6) gives very useful advice when he recommends that land after an attack should be deeply ploughed and well rolled and that no crop liable to attack should be put in directly afterwards. But this last recommendation needs explaining in the light of the fact that this weevil does not infest successive crops but breeds in cruciferous crops during one particular season in the year. His suggestion to plough in deep infested stalks may not be altogether effective as it has been noted that grubs in stalks buried 8 ins. deep are able to transform.

The recommendations that are suggested as a result of the present investigation are:

1. Root out all over-wintered infested stalks of crops like brussels sprouts, kales and cabbages, by the beginning of March and of spring cabbages as early as possible, shake off the earth from the roots and stack them up in large heaps and when dry burn them. Never leave infested stalks longer than can be helped in the soil or when they have been pulled out, scattered about, or in small lots.

2. Plough the land deep immediately the infested crop has been removed. This will crush and destroy a large number of cocoons with the pupae within.

3. Avoid in the next autumn a crop liable to attack. It is not necessary to avoid such a crop in spring or early summer as the pest does not attack cultivated crucifers at this time, but only crops grown from about the middle of July and through the autumn. At this time the land should be put under some crop that is immune.

4. Destroy all charlock (*Sinapis arvensis*) and hedge-mustard (*Sisymbrium officinale*) as the adult beetles feed on the foliage and flowers of these. The race of *C. pleurostigma*, which breeds in spring in charlock, is not infectious to cultivated crucifers, but it is possible that some of the beetles emerging very early in the year may attack in autumn some of the crops; besides it is known that charlock is a host of the fungus *Plasmodiophora Brassica* Wor. which causes the "club-root" in many crucifers.

VI. BIBLIOGRAPHY.

(1) CURTIS, J. (1859). *Farm Insects.*
(2) MASSEE, G. (1915). *Diseases of cultivated plants and trees.*
(3) ORMEROD, E. A. (1881). *Manual of injurious insects.*
(4) RITZEMA BOS, J. (1891). *Tierische Schädliche und Nützliche.*
(5) TASCHENBERG, E. L. (1879). *Praktische Insekten-Kunde.*
6) THEOBALD, F. V. (1907). *Report on Economic Zoology.*

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STUDIES IN BACTERIOSIS. IX

"STREAK DISEASE OF BROAD BEANS"

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(With Plates VIII—X.)

SYMPTOMS OF THE DISEASE.

THIS disease is characterised by chocolate spots on the leaves, broad sunken chocolate streaks with a bronze lustre on the stems, and similar coloured patches on the pods. The leaf spots are often very numerous and cover a large area of the leaf (Pl. VIII, fig. 1); the stem lesions are of varying size from half an inch to four or five inches, they often extend the length of an internode and run intermittently throughout the length of the stem, recalling very strikingly the stem lesions in "stripe" of tomatoes and "streak" in potatoes and sweet peas. The trouble often starts at the base of the plant and works upwards, but sometimes the whole plant seems to be attacked suddenly and uniformly; in the former case soil or seed infection, and in the latter air infection, would seem to be indicated. The lower leaves fall prematurely and the stems are left with a small number of unhealthy leaves at the top with only a few pods of small size and these often much spotted.

OCCURRENCE OF THE DISEASE.

The disease was very troublesome upon field beans during the summer of 1920. It commenced in May in South Wales and rapidly spread, or seemed to spread, eastward. It was observed by one of the authors in Devon during June, in Sussex in July, and was reported successively from Buckinghamshire, Cambridgeshire and Lincolnshire. It was undoubtedly very general throughout a large part of England and the evidence of an easterly spread, which would indicate wind dispersal, is not sufficiently strong to be considered reliable. When this investigation began about the middle of June the disease was well advanced, having appeared in Carmarthen some six or eight weeks earlier. In one case, beans planted in October, 1919, first showed signs of disease on May 25th, small purplish brown spots being observed on the leaves of plants then about five feet high. When next observed, June 8th, the plants were largely defoliated, the remaining leaves showing a good deal of blackening. The weather at the time of the onset was said to have been hot and thundery, and when these fields were examined on July 10th by Mr Wiltshire of the Long Ashton Experiment Station, the canes in the central portion of the field were beaten down by rain, and all the leaves were defoliated except a bunch at the top of each stalk. The whole crop was rapidly destroyed by *Botrytis* which, in all cases, seems to follow closely upon the streak disease.

In many instances it was observed that the plants in the outer portions of the fields were less severely attacked than those in the centre; being more exposed the outer portions would naturally tend to be under drier conditions.

On a farm near Sidmouth in South Devon the onset of the disease occurred during three days of dense fog; in this case the whole field suffered equally and in three weeks the plants were practically stripped of their leaves.

It is probable that the disease is always present to some slight extent upon winter beans and that unfavourable weather conditions were responsible for the extensive epidemic of 1920. One farmer could recall a similar destruction of his beans some thirty years ago and that he had at that time attributed the trouble to lightning injury. Undoubtedly heavy moisture, rain or fog, and high temperature are the conditions which favour the spread of this disease. The freedom from the disease exhibited by the crops of the abnormally dry summer of 1921 is strong evidence in this connection; the disease did, in fact, make its appearance in a field near Bristol which had borne a badly diseased crop the previous year but the plants spontaneously recovered and later not a trace of the disease was to be found.

The losses incurred during 1920 were very heavy, on many farms the crops were not considered worth harvesting, on others the estimated loss was fully 75 per cent. It is probably a low estimate to give the total loss for southern England as 40 per cent. or 50 per cent.

PATHOLOGICAL ANATOMY.

Microscopical examination of the lesions or streaks on the stem shows in many cases destruction of the epidermal and the sub-epidermal cells of the cortex, no effect being found in the deeper tissues. In other cases the pith is browned for a considerable distance up the stem, and from the pith the disease can be traced by way of the medullary rays to the cortex, and through this it passes to produce lesions under the epidermis as stated above. The spots on the leaves extend through the entire thickness of the leaf or may be confined to the epidermal and subepidermal cells only, or, again, only the guard cells of the stomata are found to be discoloured. The first sign of disease of a palisade cell is a reddening of the cell contents, next these appear to be in a state of disorganisation and finally to be reduced to a disintegrated mass held together by a reddish-brown gummy substance. The cell walls become swollen, the middle lamella partly dissolve and the cells collapse. The walls and the granular gummy contents stain strongly with carbol-fuchsin, an effect which is very commonly produced by bacterial action.

The causal organism.

Attempts to isolate a bacterial parasite were not always successful, many of the lesions seemed to contain no living organism, others gave only the usual soil saprophytes, but in about 20 per cent. of all such attempts a yellow organism appeared which proved to be *Bacillus lathyri.*

The symptoms of the disease fully accord with the attack of this organism on other hosts. It has been shown by Manns and Taubenhaus (1), Manns (2), and recently confirmed by Paine and Bewley (3), to produce streak disease of sweet peas and other legumes. The symptoms in these cases and in the stripe disease of tomato (4), which is also caused by *B. lathyri*, are exactly the same as far as stem lesions are concerned as those described above.

B. lathyri was isolated from leaves of beans from Carmarthen, Sidmouth and Bristol. Attempts to obtain the organism from the brown pith of diseased stems and petioles failed in every case. It was also obtained from stem lesions of clover plants from a field near Bristol adjacent to a badly attacked crop of beans.

Along with *B. lathyri* a number of yellow organisms were isolated and many of these appeared to be so similar to it, yet differing in pathogenicity and certain other characters, that a special study of them was made in order to determine whether or no they were non-virulent strains of the same organism. The results of this investigation are contained in a separate paper (5).

INOCULATION EXPERIMENTS.

Preliminary infection experiments made in June, 1920, showed that the disease could be reproduced by grinding up some of the spotted leaves in water and pricking this suspension into healthy bean leaves. In five days the tissue round the point of infection had a typical chocolate brown colour to a depth of 1–2 mm.; the controls were all negative. Following these, however, all experiments conducted during the summer of 1920 were spoiled by an extraordinary prevalence of the disease making it impossible to keep the control plants uninfected.

In April, 1921, an extended series of hypodermic needle infections was made with three strains of *B. lathyri* (one from tomato and two strains 33 and 45 c isolated from beans) and with two strains of a yellow organism (designated 20 and "b") which gave all the reactions of *Pseudomonas Phaseoli*, and which was frequently isolated from diseased plants along with *B. lathyri*. Three plants were inoculated with each of these five strains and sets of three were inoculated with mixtures of the *B. lathyri* strains and the strains 20 and "b" in all possible combinations to see whether this organism had any part in the etiology of the disease. Twelve control plants were inoculated with boiled suspensions of the various organisms. All the plants were cut longitudinally through the point of inoculation at the end of three weeks. The controls showed no sign of disease and the wound made by the needle was only just perceptible.

B. lathyri (tomato strain). Plant (1) red spot, 3 mm. diameter round the hole; vascular bundles brown for a length of 3 inches.

Plant (2), slight brown mark ½ inch up the stem. No interior lesion.

Plant (3), no disease.

B. lathyri (strain 45 c from *Vicia*). Plant (1), brown streak running up the v.b. for 4 inches then passing out through the cortex and causing a reddish-brown streak on the epidermis 2 inches long, ending at the petiole above.

Plant (2), same as (1).

Plant (3), brown streak passing up the v.b. and out through the cortex to produce exterior brown streaks intermittently up the stem. The brown v.b. traceable to a petiole 9 inches up the stem, petiole and leaves spotted with red-brown spots; leaves on the other side of plant not spotted.

B. lathyri (strain 33 from *Vicia*). Plants (1) and (2) show typical red sunken marks 3 mm. diameter on the exterior at the point of inoculation. The v.b. is brown 6 inches up the stem and traceable to angular red streaks on the epidermis as above.

Plant (3) shows very little effect in the stem (very woody stem) but the v.b. is brown 4 inches up and traceable to the petiole above.

Strains 20 *and "b."* All six plants gave negative results, and the plants inoculated with mixtures of these with *B. lathyri* showed no more extended disease than those with *B. lathyri* alone.

Other inoculation experiments were made with combinations of *B. lathyri* and various white organisms which appeared to be associated with *B. lathyri* in the host plant but there was no evidence that these white organisms aided infection in any way.

Following the successful inoculation experiments detailed above came a period throughout the entire summer of 1921 during which numerous abortive attempts to produce disease of beans growing in the open by inoculation with the strains 33 and 45 c caused doubt as to whether the causal organism had really been obtained. All these doubts, however, were dispelled in September when the same strains which had been used all through the summer gave very positive evidence of infection. The weather conditions at this time led to very heavy dews in the early mornings followed by hot days, conditions which seem to favour the development of the disease. The previous failures to produce disease are doubtless accounted for by the extreme drought of the summer months, the plants probably rarely—if ever—attained full turgidity and hence enjoyed immunity against the bacterial attack.

A few examples of the September experiments may be given.

I.

8. ix. 21. *Leaf inoculation.* A drop of fluid culture was placed on the leaf and the surface gently pricked with a glass needle. Controls similarly inoculated with boiled culture.

Twelve pricks were made with each of three strains of *B. lathyri.*

13. ix. 21. Twenty-one chocolate spots well developed; lesions extend right through thickness of leaf; most of the remaining points show a slight reddened area round the wound; a few are negative, these being situated at the top of leaf where they would tend to dry up more quickly than at the base. Controls all negative.

II.

23. ix. 21. *Leaf inoculations* with organisms isolated from the previous experiment. One hundred and ten inoculated spots and 130 control spots with boiled culture.

28. ix. 21. Eighty-three large chocolate spots about ½ cm. diameter. Control pricks all negative.

3. x. 21. No further development. Two leaves photographed (see Pl. VIII, fig. 2).

III.

8. ix. 21. *Stem inoculations.* Six plants grown outdoors inoculated with the same strains of *B. lathyri* as were used in Exp. I. Inoculation by a hypodermic needle in the axil between the stem and a leaf. Six control plants similarly inoculated with boiled culture.

13. ix. 21. *Controls.* Point of inoculation healed, no reddened area. Pith browned where the needle had entered.

Inoculated plants. Four plants show a reddish-brown lesion about ½–1 cm. diameter round the point of infection with a few streaks running up the stem to a height of 4 cms. Otherwise the exterior of the stem is unmarked. The pith is browned for a distance of 15–18 cms. up the stem, no lesions in the cortex. The other two plants show the same exterior lesions on the stem and local browning of the pith but no spread up the pith.

IV.

15. ix. 21. *Leaf inoculation* with Strain 70. One hundred spots, all negative. The authors would draw special attention to the failure of this strain to produce disease since careful comparison of Strain 70 with other strains of *B. lathyri* proved it to be in other respects identical. Detailed description of these comparative experiments are to be found in the communication which follows (see p. 204 of this Number).

Inoculation of Broad Bean Cotyledons.

The seed was sterilised externally by means of mercuric chloride and the testa slit open with a sterile knife, the cotyledons were placed in moist chambers and inoculated at the centre with a drop of a suspension of *B. lathyri*, the surface being gently scratched with a sterile needle. Controls were prepared in exactly the same way with boiled suspensions of the organism.

Experiments were made with ten different strains of *B. lathyri* and in every case resulted in browning and destruction of the tissue to a depth varying with the virulence of the strain from 1 mm. to the entire thickness of the cotyledon; the controls were practically unaffected (see Pl. VIII, fig. 3). Where the virulence of the strain was comparatively weak the diseased area was surrounded by a darker brown zone of cork.

Similar attempts at inoculation were made with the other organisms, *e.g.* strains 10 and 20 of *P. Phaseoli* and various white organisms mentioned above. All these attempts failed to produce any symptoms of disease.

Evidence of rapid desiccation of B. lathyri *in lesions.*

The fact that attempts to isolate the causal organism from lesions in the pith and from many spots on the leaves proved abortive suggested that the organism possessed small power of resistance to dry conditions. Bean plants growing in the open were sprayed with a suspension of *B. lathyri* on 28. vi. 21. Typical red brown spots developed on the under surface of the leaves. On 12. vii. 21 attempts to isolate the organism from these spots failed entirely.

In a second experiment on 8. vii. 21 leaves were infected by prick inoculation. On 15. vii. 21 six spots cut out with sterile instruments but without surface sterilisation were dropped into broth tubes. After 24 hours' incubation a loopful of the broth was plated. Four of the tubes gave *B. lathyri* and two failed to do so. On 22. vii. 21 six spots were again cut out and treated as before, with the results that two gave pure cultures of *B. lathyri* and four failed. In a third experiment on 8. ix. 21, 20 days after inoculation plating from one spot gave no growth of *B. lathyri* and from a second spot only 12 colonies of the causal organism developed from the whole of the diseased tissue of the spot.

The restriction of the disease to small areas on the leaves and to narrowly circumscribed streak lesions on the stems is therefore fully explained by destruction of the organism by desiccation of the diseased tissue.

THE SPREAD OF THE DISEASE.

As stated earlier the spread of this disease is mainly the result of certain favourable atmospheric conditions. Wind dispersal of the organism is undoubtedly a strong possibility; the organism thus carried falling upon the surface of the plant wetted by rain, dew or fog during warm weather has ample opportunity to multiply and produce stomatal infection.

Many of the diseased crops were smothered with aphides and these can undoubtedly carry the organism from plant to plant as has been shown by allowing an aphis to walk across the surface of an agar plate. After removal of the aphis and incubation of the plate many yellow colonies developed and these were proved to be those of *B. lathyri.*

Aerial infection, though very prevalent, is not the only means of spread of disease. It is perfectly obvious to the observer that infection of the plant must often arise either from the soil or the seed. Field notes made at Carmarthen and elsewhere established the fact that the disease often starts from the base of the plant and works upwards. On one small garden plot which held about 200 plants of broad bean a *single* individual was typically diseased, smothered with brown spots and stem lesions from top to bottom. A photograph of this plant appears in Pl. IX, fig. 4. The neighbouring plants were normal, so obviously this must have been a case of seed infection, or an isolated case of wound infection below the soil level, which is less probable.

Bruchus rufomanus *as a cause of seed infection.*

Some bean seed from a badly diseased crop of the previous year was obtained in March, 1921, and many of the beans were found to be bored by the bean beetle, *Bruchus rufomanus*. These were separated from sound beans and soaked overnight in water, the water containing bored beans became quite turbid while that containing sound ones remained almost clear. The liquids were plated out and from the turbid water an almost pure plate of yellow organisms resulted. These had all the appearance of colonies of *B. lathyri*. The experiment was repeated with seed from one of the well-known seedsmen, a sample which contained a large number of beetle-bored seed. The seed was poured straight from the bag into a sterile dish from which bored and sound beans were selected and placed in sterile vessels containing 50 c.c. of sterile water as under:

Beaker A. 12 sound beans.
B. 12 beetle-bored beans.
C. 8 sound plus 4 beetle-bored beans killed by boiling.
D. 12 beetle-bored beans boiled for five minutes.

After 24 hours at room temperature a loopful of water from each was plated on agar with the following result:

Beaker A. 32 colonies developed all yellow and proved to be *B. lathyri*.
B. 760 yellow colonies, *B. lathyri*.
460 white colonies, *B. subtilis* type.
C. 34 yellow colonies, one white.
D. 2 colonies of penicillium only.

This experiment was repeated with beans from another source.

Ten sound beans gave one white colony.

Ten beetle-bored beans gave 16 white colonies; two Penicillium colonies.

No colonies of *B. lathyri* developed from these beans, but, as the following table shows, streak disease developed to a certain extent when the bored beans were planted.

The table below shows the result of planting beans from these two sources designated *A* and *B*.

No. of beans planted	Source	Condition of beans	No. germinated	% germination	Condition of plants	% of diseased plants
50	A	Sound	44	88	35 healthy 9 slightly streaked	20
50	A	Beetle-bored	6	12	1 healthy 5 streaked	83
15	B	Sound	15	100	All healthy	0
15	B	Beetle-bored	14	93	12 healthy 2 diseased	14
10	B	Spotted with chocolate spots (see Pl. X, fig. 5)	10	100	8 healthy 1 slightly diseased 1 badly ,,	20

The "spotted" beans referred to above had several chocolate spots on the testa, these extended through the testa but no lesions were observed on the cotyledons beneath. The plant arising from one of these seeds and said to be "badly diseased" was interesting in its development; the first shoot came up only one inch above the ground, had a wide chocolate coloured streak up one side and several smaller lesions, growth stopped and the shoot died. A second shoot was put out and grew to a height of four inches, it also showed disease in a broad streak 2–3 mm. wide and 3 cm. long starting at the junction with the cotyledon. This shoot died back and a third one developed, grew to a height of nine inches and appeared to be perfectly healthy when the plant was dug up for examination. The tap root was unmarked but four of the secondaries were streaked and the cotyledons were spotted throughout with chocolate-coloured lesions (Pl. X, fig. 6). *B. lathyri* was isolated from the cotyledons and from the second shoot. The entire third shoot was ground with sand and water and plated but no *B. lathyri* developed, it therefore appears that this shoot had outgrown the disease. Reference may be made here to a means of control of tomato "stripe" caused by the same organism where by the cutting off of the diseased stems laterals often give rise to healthy plants.

METHODS OF CONTROL.

The "streak" disease of beans is one which is always present amongst field beans but usually to such a small extent that it need not be contemplated seriously. The disease assumes an epidemic form only under exceptional climatic conditions; these exceptional conditions prevailed in 1920 and gave rise to a bad epidemic. A repetition of the trouble was expected in 1921 as the 1920 seed was badly infected; inspection of the crops showed, however, only an insignificant amount of the disease in this extremely dry year.

Where disease has occurred seriously it would be advisable, before another crop of beans is carried, to dress the land well with potash, since it has been shown that this treatment has successfully checked the ravages of the bacillus upon tomato plants, and there is every reason to expect that it would be equally effective in controlling the disease of beans. Further, it would be well to examine the seed carefully and to reject any showing an excessive amount of boring by beetles, and to sterilise the seed by soaking in weak lysol or formaline.

When the disease appears in the crop and there is at the same time an infestation of the black fly, spraying to destroy these would almost

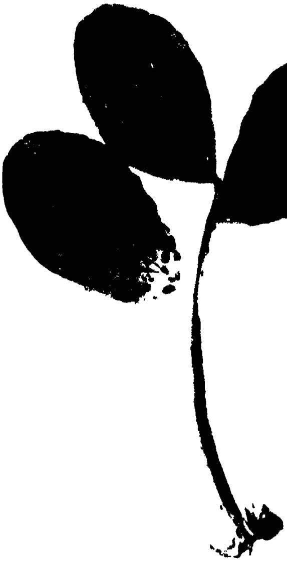


Fig. 1.

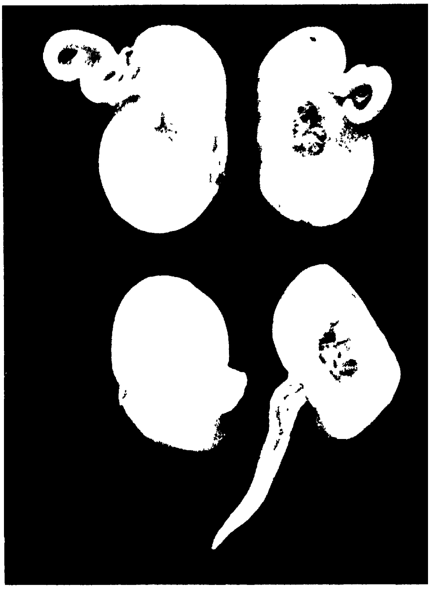


Fig. 3.

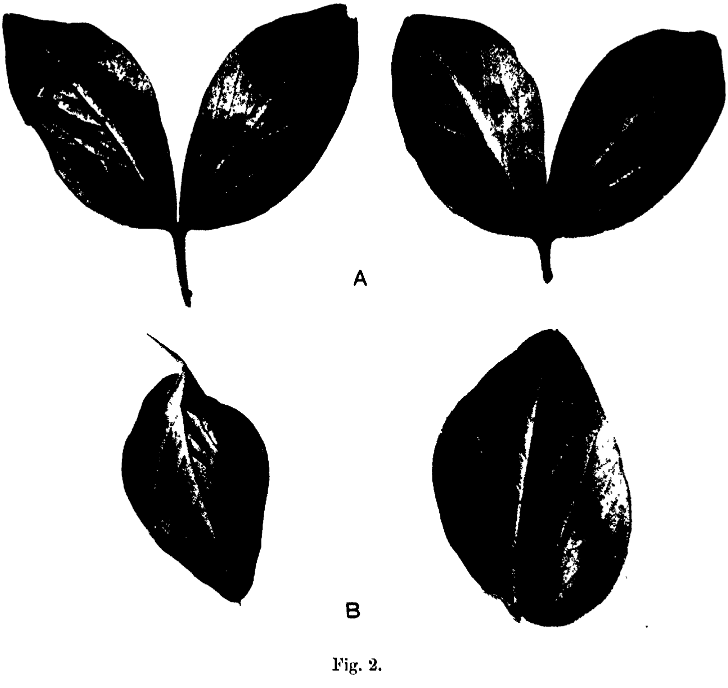
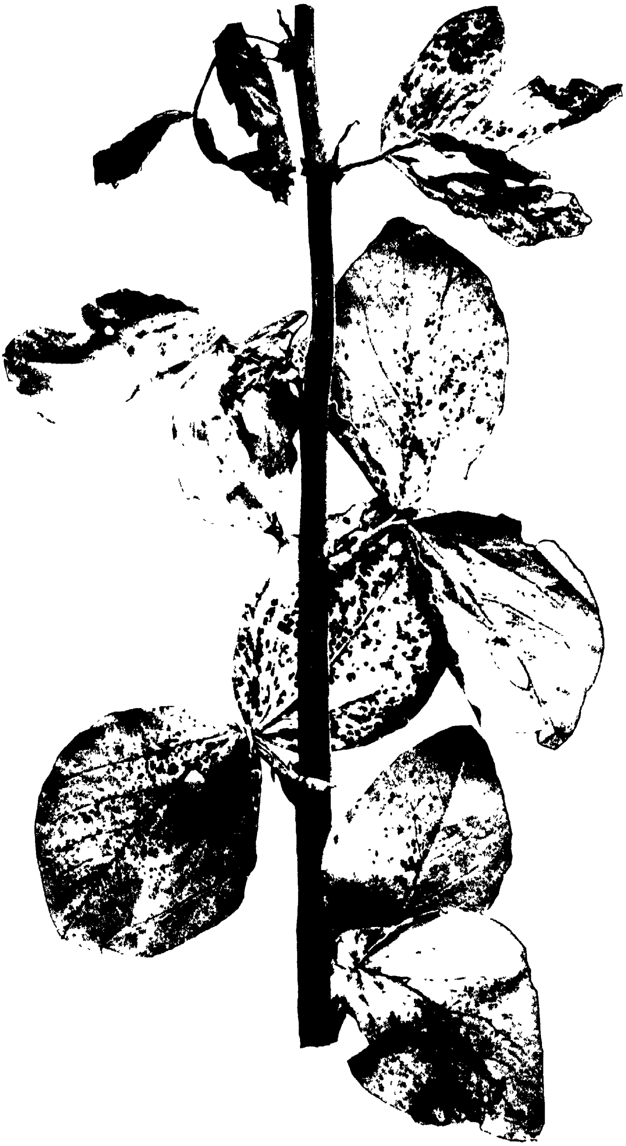


Fig. 2.

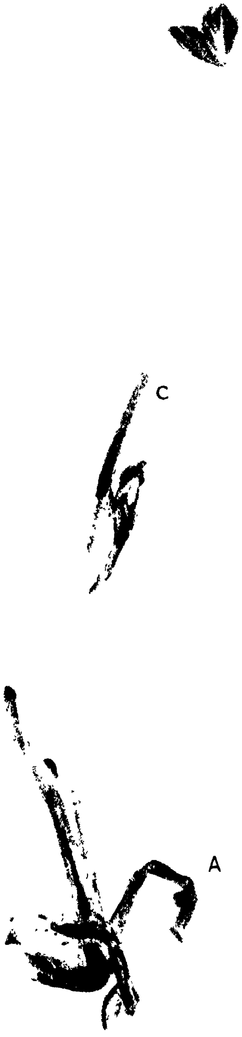
PAINE & LACEY.—Studies in Bacteriosis. IX. "Streak Disease of Broad Beans." (pp. 194—203.)

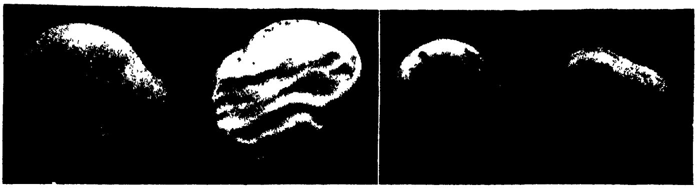


[From the *Journal of the Ministry of Agriculture* by permission of the Ministry of Agriculture and His Majesty's Stationery Office]

Fig. 4.

PAINE & LACEY.—STUDIES IN BACTERIOSIS. IX, "STREAK DISEASE OF BROAD BEANS." (pp. 194—203.)





Figs. 5 and 6.

PAINE & LACEY.—Studies in Bacteriosis. IX. “Streak Disease of Broad Beans.” (pp. 194—203.)

certainly save the crop to a considerable extent. While in the past spraying for black fly has not commended itself to the grower, recent improvements in the manufacture of insecticides have made possible the destruction of this pest.

In conclusion the authors wish to express their thanks to Messrs A. D. Cotton, S. P. Wiltshire and W. H. Broadbent of the Ministry of Agriculture for notes on the disease as it occurred in the field and to Mr J. C. F. Fryer for notes on the habits of the bean beetles.

SUMMARY.

The "streak" disease of beans (Chocolate Spot Disease) is caused by *B. lathyri*.

Moist and warm conditions are responsible for the appearance of the disease in epidemic form.

The disease is spread by wind, black fly, and the bean beetle.

Methods of control are suggested.

REFERENCES.

(1) MANNS, T. F. and TAUBENHAUS, J. J. (1915). *Gard. Chron.* 53, p. 215.
(2) MANNS, T. F. (1915). Delaware College Agr. Exp. Sta. *Bull.* 108.
(3) PAINE, S. G. and BEWLEY, W. F. (1923). *Annals of Applied Biology*, x, 89.
(4) —— (1919). *Ibid.* VI, 183.
(5) PAINE, S. G. and LACEY, M. S. (1923). *Annals of Applied Biology.* x, 204.

EXPLANATION OF PLATES VIII—X

PLATE VIII

Fig. 1. Showing the characteristic chocolate-coloured spots on the leaf, the large patches on the margins are due to secondary infection by *Botrytis*.

Fig. 2. A. Showing spots as the result of prick inoculation of bean leaves with *Bacillus lathyri*. B. Showing needle prick marks in control leaves.

Fig. 3. Showing result of inoculation of bean cotyledons with *B. lathyri*; inoculated half of bean on the right, control half on the left.

PLATE IX

Fig. 4. Showing the characteristic streak lesions on the stems and chocolate-coloured spots on the leaves.

PLATE X

Fig. 5. "Spotted" beans referred to in the table on p. 201.

Fig. 6. Plant raised from a spotted bean. A. First shoot (diseased). B. Second shoot (diseased). C. Third shoot (healthy).

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STUDIES IN BACTERIOSIS. X

"THE USE OF SERUM-AGGLUTINATION IN THE DIAGNOSIS OF PLANT PARASITES"

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INTRODUCTION.

During the course of investigations of the "Stripe" disease of tomato (5) and of the "Streak" disease of beans (6), both of which are caused by the same parasite, *Bacillus lathyri*, certain other yellow bacteria were frequently isolated at the same time as this organism. Chief amongst these was one which bore a certain superficial likeness to *B. lathyri* but differed from it in its physiological behaviour; in fact it gave all the characters of *Pseudomonas Phaseoli*. These two organisms possess many distinguishing features, *e.g.* in their sugar fermenting properties and in their power of liquefying gelatine. However, Harding and Morse (2) showed that various strains of *Bacillus carotovorus* might differ widely in fermentation reactions, but on simultaneous cultivation under identical conditions such differences disappeared, and the sugar fermentations could be relied upon for diagnostic purposes if certain previous cultural precautions were taken. Later, Morse (4) showed that the power of liquefaction of gelatine was also liable to change under similar treatment. With this in mind it seemed possible that the organism which appeared to be *Pseudomonas Phaseoli* might, in reality, be a degenerate form of the species *B. lathyri*. Several strains of both organisms were therefore "trained" simultaneously in bouillon by daily transfers until the tenth day when they were plated on bouillon agar, and subsequently inoculated into the various sugar and other media generally used for diagnosis. The results given in Table II (p. 207) showed that even after such treatment the organisms retained their individual characters.

In order further to verify these results it seemed advisable to have recourse to animal inoculation in the hope that specific agglutinins would be produced. This is not the first instance of the use of serological tests in connection with plant organisms. Zipfel (8) in 1912 and Klimmer and Krüger (3) in 1914 were able to group various strains of *Bacillus radicicola* according to their serum agglutination phenomena and Doidge(1) in 1917 found that the serum of a rabbit inoculated with *Bacillus citrimaculans* agglutinated that organism in dilutions down to 1–2000.

EXPERIMENTAL.

The serum used in this work was prepared by Dr St John Brooks of the Lister Institute of Preventive Medicine to whom, and to his assistant, Miss M. Rhodes, the authors desire to express their sincere thanks.

Three organisms were employed for inoculations:

(1) Strain 33, *Bacillus lathyri* from Bean.

(2) Strain 20, *Pseudomonas Phaseoli* from Sweet Pea.

(3) Strain 40, *Aplanobacter Michiganense* E.F.S. from Tomato.

1 c.c. of a thick emulsion of the several organisms in normal saline was injected intravenously into young rabbits. No pathogenic symptoms were observed in any case so the dose was repeated a week later. At the end of the second week a sample of serum from each rabbit was collected and tested for agglutinins; strain 33 agglutinated with its serum diluted 1 in 2000, strain 20 with its serum diluted 1 in 200, but strain 40 failed to show agglutination. A third dose was given to rabbits "20" and "40" a week later, and a fourth dose to rabbit "40" after the lapse of another week. Serum was collected from rabbit "33" four weeks, and from rabbits "20" and "40" five weeks after the first inoculation. At this time the titre of 33 and 20 was 1 in 2000 in each case, but 40 again failed to agglutinate in any dilution.

Method of agglutination. Dilutions of the serum were made with water to 1–10, 1–50, 1–100, etc. 0·5 c.c. of an emulsion in water from a 24-hours' culture on agar was placed in the tubes and 0·5 c.c. of the diluted serum added, thus giving dilutions 1–20, 1–100, 1–200, etc. In the control tubes 0·5 c.c. of water took the place of the serum. The tubes were immersed in water at 30° C. and incubated for four hours. Observations were then made and verified the next morning after standing in the cold overnight.

While all strains of *B. lathyri* exhibit marked agglutination with the 33 serum, the agglutinating power of the serum is noticeably different for different strains. This is not attributable to the source from which the strains were derived. Numbers 2 and 3, for instance, were isolated from the same sweet pea plant, they were kept as separate strains on account of a slight difference in the colour developed in the growth on agar; they gave identical fermentation reactions, yet they show marked difference in agglutination.

Table I. *Showing agglutination of various strains of* Bacillus lathyri.

+ + indicates strong and + slight agglutination.

No. of strain	Host plant	Serum dilutions				
		1–500	1–800	1–1000	1–2000	1–2500
1	Broad bean	+ +	+ +	+	—	—
2	Sweet pea	+ +	+ +	+ +	+	—
3	„	+ +	+ +	—	—	—
4	Inoculated broad bean	+ +	+ +	—	—	—
5	„ „	+ +	+ +	—	—	—
6	Broad bean	+ +	+ +	+ +	+	—
7	„	+ +	—	—	—	—
8	Clover	+ +	+	—	—	—
21	Washings of beetle-bored beans	+ +	+ +	+ +	+	—
26	Washings of uninjured beans, from the same packet	+ +	—	—	—	—
45	Broad bean	+ +	+ +	+ +	+	—
18	Sweet pea	+ +	+	—	—	—
33	Broad bean	+ +	+ +	+ +	+ +	+
Laboratory stock culture	Tomato	+ +	+ +	+ +	+	—
70	Broad bean	+ +	+ +	—	—	—

Again, *B. lathyri* from a tomato plant agglutinated at a dilution of 1 in 2000, while strain 7 from a broad bean, and thus from the same host as the strain used in preparation of the serum, agglutinated at a much higher point, namely, 1 in 500.

Moreover, the variation shown throughout the table cannot be attributed to the length of time the strains have been cultured on artificial media, for strains 2 and 3 were isolated at the same time and underwent exactly parallel treatment; this is also true of strains 21 and 26, while strains 1, 6 and 7 were isolated within a week or two of each other and show considerably different degrees of agglutination. No explanation of this variation is at present advanced. It is interesting to note, however, the occurrence of serological strains amongst plant pathogens which seem to be exactly similar to those which occur amongst the human pathogens. Some very interesting results with regard to serological strains in the enteric and dysenteric groups of bacteria have recently appeared in a preliminary communication by Walker(7). Agglutinable and dys-agglutinable forms may be obtained at will from one and the same strain of a bacillus and both may revert or may be converted mutually the one into the other.

In Table II are given the results of comparative experiments with various strains of *Bacillus lathyri, Pseudomonas Phaseoli* and *Aplanobacter dissimulans* and *michiganense.*

Table II.

	Strain	Lowest dilution at which agglutination takes place		Sugar fermentation with production of acid in			Nitrate reduction	Diastatic action	Liquefaction of gelatine	Motility
		Serum 33	Serum 20	Glucose	Lactose	Sucrose				
33	*B. lathyri* from broad bean	1–2500	1–20	+	—	+	+	+	Very slow	Motile by peritrichous flagella
Stock culture	*B. lathyri* from tomato	1–2000	—	+	—	+	+	+	”	”
2	*B. lathyri* from sweet pea	1–2000	1–20	+	—	+	+	+	”	”
26	*B. lathyri* from winter bean	1–500	1–20	+	—	+	+	+	”	”
5	*B. lathyri* from broad bean	1–800	1–20	+	—	+	+	+	”	”
70	” ”	1–800	1–20	+	—	+	+	+	”	”
94	*B. lathyri* (?)	1–100	1–100	—	—	—	—		None	—
20	*P. Phaseoli* from sweet pea	—	1–2000	Slight after 5 days	—	—	—	+	Rapid	Motile by single polar flagellum
*b**	*P. Phaseoli* from broad bean	1–20	1–20	Slight after 5 days	—	Slight after 5 days	—	+	Not so rapid as 20 but not so slow as *B lathyri*	”
10	*P. Phaseoli* from Phaseolus leaf	—	1-100	—	—	—	—	+	Rapid	”
9	*A. dissimulans* from Phaseolus leaf	—	—	Very slight after 5 days	—	Very slight after 5 days	—	+	”	Not motile
Stock culture	*A. dissimulans* from tomato	—	—	Very slight after 6 days	Very slight after 9 days	Very slight after 6 days	—	+	”	”
40	*A. michiganense* from tomato	—	—	Slight after 13 days	Slight after 13 days	Slight after 13 days	—	+	Very slow	”

* Control with normal rabbit serum.

In the above table it will be seen that *Bacillus lathyri* and the two organisms, *Pseudomonas Phaseoli* and *Aplanobacter dissimulans*, which often appear on isolation plates from tissues of plants diseased by *Bacillus lathyri*, retain their distinguishing morphological and physiological characters even after the preliminary training mentioned above (p. 204). They must therefore be regarded as distinct species. Strains 94 and *b*, however, seem to be intermediate types between *Bacillus lathyri* and *Pseudomonas Phaseoli*, this is shown in their physiological reactions and in the fact that they agglutinate equally with agglutinating sera of both species. *Bacillus lathyri* in all but one of the strains was agglutinated slightly but definitely with the serum of *Pseudomonas Phaseoli*. This seems to show a relationship similar to that existing between *Bacillus typhosus* and *Bacillus paratyphosus A* and *B* and suggests the possibility that one of the species may have arisen by mutation from the other. If such mutations occur in the tissues of the host plant an explanation would be found for a phenomenon with which the plant pathologist is very familiar, namely, that on plating from diseased tissues colonies develop which appear to be all of the same organism, yet few of these, and sometimes quite a small percentage, possess any virulence for the host plant; it is possible that the non-virulent forms are mutants of the virulent one.

The *Aplanobacters* seem to be entirely distinct from *Bacillus lathyri* showing quite different physiological behaviour and no agglutination with either sera 33 or 20 and, as stated above, even with the serum 40 prepared from *Aplanobacter michiganense*.

SUMMARY.

(1) Serum agglutination has been used in order to determine possible relationship with *Bacillus lathyri* of certain yellow organisms which occur in the tissues of plants infected by that organism.

(2) One of these gave all the reactions of *Pseudomonas Phaseoli*.

(3) *Pseudomonas Phaseoli* and *Bacillus lathyri* gave distinct cultural characters, and these were retained even after the two organisms had been "trained" by ten transfers through the same medium.

(4) The two organisms seem to be susceptible to group agglutination, *Bacillus lathyri* being agglutinated with the serum of *Pseudomonas Phaseoli*.

(5) Intermediate forms between these two organisms are found which agglutinate equally with the sera of both species.

(6) The suggestion is made that one species may have arisen in the tissues of the plant as a mutant from the other.

(7) *Aplanobacter dissimulans*, another organism often associated with *Bacillus lathyri*, appears to bear no sort of relationship with the latter.

(8) *Aplanobacter michiganense* failed to produce an agglutinating serum on inoculation into a rabbit.

REFERENCES.

(1) DOIDGE (1917). *Annals of Applied Biology*, III, 53.
(2) HARDING and MORSE (1909). *New York Agric. Exp. Sta., Tech. Bull.* No. 11.
(3) KLIMMER and KRÜGER (1914). *Centralbl. f. Bakt.* 2 Abth. XL, 256.
(4) MORSE (1917). *Jan. Agric. Research*, VIII, 79.
(5) PAINE and BEWLEY (1919). *Annals of Applied Biology*, VI, 183.
(6) PAINE and LACEY (1923). *Ibid.* X, p. 194.
(7) WALKER (1922). *Proc. Roy. Soc. B.* 93, p. 54.
(8) ZIPFEL (1912). *Centralbl. f. Bakt.* 2 Abth. XXXII, 97.

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ON THE RELATIVE IMPORTANCE OF CERTAIN COMMON GRASSES AS HOST PLANTS OF *OSCINELLA FRIT*, LINN.

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Previous experiments (1) have indicated that under artificial conditions the frit fly exhibits a certain degree of preference for *Arrhenatherum* species among grasses. In order to determine what occurred under natural conditions (for the fly) in the field, the seed of 12 different species of grasses, known to be liable to infection, was sown in duplicate on plots of one square yard, the plots being situated ten yards from an oat crop. Sowing was deferred until 20. iv. 22 to ensure infestation; the unfavourable conditions for germination prevailing at this time and also the varying quality of the seed greatly affected the numbers of plants obtained. The plants were collected on 11. vi. 22, being sampled at hazard where numerous, and were examined in the laboratory for signs of frit attack. Flies were reared from the puparia[1] obtained during this scrutiny and as no species other than *O. frit* emerged, it seems legitimate to assume that all the larvae also were those of *O. frit*.

	Series I				Series II				
Host plant*	No. of plants	No. of puparia	No. of larvae	Infestation per 100 plants	No. of plants	No. of puparia	No. of larvae	Infestation per 100 plants	Infestation mean value
Arrhenatherum avenaceum	66	16	36	78·8	56	22	26	85·8	82·3
A. avenaceum var. *bulbosum*	60	22	24	76·7	50	12	30	84·0	80·3
Hordeum murinum	42	10	32	100·0	40	8	14	55·0	72·5
Agrestis myosuroides	40	6	24	75·0	40	11	16	67·5	71·2
Lolium italicum	88	10	24	37·6	54	10	26	66·7	52·1
L. perenne	51	2	2	7·8	70	—	4	5·7	6·7
Festuca pratensis	12	—	1	8·3	10	—	—	—	4·1
Dactylus glomerata	36	—	2	5·6	21	—	—	—	2·8
Holcus lanatus	50	—	—	—	43	—	—	—	—
Bromus sterilis	47	—	—	—	37	—	—	—	—
Oat crop (adjacent—sown, March 17th)	49	15	18	67·3	—	—	—	—	67·3

* *Hordeum pratense* and *Avena flavescens* failed to grow.

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[1] Only 34·5 per cent. of the larvae had pupated by 11 vi. 22.

The data obtained from these plots are collected in the preceding table. The column showing the number of puparia discovered includes the stems obviously drilled by the larvae but not containing either stage at the time of examination. These were very few in number, but may be the cause of slight inaccuracy since each such stem may have contained two or more larvae. As the influence of the grass hosts on the field population seems likely to become an important factor, the comparison between the grass species has been based on the infestation per hundred plants.

These figures are comparative only in the sense that they refer to plants produced from seed germinating about the same time[1]. There seems to be no information readily available as to when germination of the seed of wild grasses occurs in nature.

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[1] Although it is notorious that the liability of oat plants to infestation is in some degree correlated with growth, the factors determining differences in susceptibility to attack have not yet been investigated. In 1922 an experiment, as yet unconfirmed, conducted to ascertain the relation between age of plant and intensity of attack yielded a somewhat unexpected result and is of interest in this connection.

Oat seed was sown in 9″ pots, every seven days from April 6th to May 4th inclusive, the resulting plants being protected from the fly in a cold greenhouse. On May 10th, five pots of each series were distributed regularly under a large muslin outdoor cage and on May 16th the cage was infected with 200 flies from the field, these flies being allowed to remain in association with the plants for a period of fifteen days. On May 10th the total numbers of plants in the five series were in order of age, (*a*) 117, (*b*) 87, (*c*) 85, (*d*) 73, (*e*) 68, while the average growth in centimetres was (*a*) 19, (*b*) 15, (*c*) 11, (*d*) 6, (*e*) 0·5.

Examination on July 18th for evidence of larval working, showed that the distribution of the attack, as denoted by the number of bored stems in each pot in each series, was (*a*) 0, 1, 0, 3, 0, (*b*) 0, 1, 3, 1, 1, (*c*) 3, 4, 4, 1, 1, (*d*) 1, 0, 0, 1, 8, (*e*) 0, 0, 1, 0, 0, *i.e.* the percentage infestation in order of age was 3·4, 6·9, 15·3, 13·7 and 1·5 respectively. The comparatively high value obtained in the fourth series is dependent on an abnormal infestation in one pot and should probably be of the order of that of the second series. It is possible that either morphologically or physiologically the plants in this one pot belonged to the third series.

The interest of these data lies in the fact that intensity of infestation was not inversely related to age, there being a period, early in the cycle certainly, when the main stem was in the most susceptible condition. Now the plants appeared above the surface of the soil on the average in seven days from the date of sowing, thus the ages of the plants in each series at the time of infection (May 16th) were (*a*) 33, (*b*) 26, (*c*) 19, (*d*) 12 and (*e*) 5 days respectively. The third series being most severely attacked it would appear that the most susceptible period in the early part of the plant cycle occurred about the 19th to the 26th day from the date of appearance above soil, *i.e.* while the plants were growing in height from 15 to 25 cm. During the infection period the first series had tillered but the tillers were only approaching susceptibility as the infestation was slight; the second series had practically passed out of the most susceptible stage; except for the abnormally infested pot, the fourth series was not heavily infested, although the susceptible stage fell entirely within the infection period—this stage was reached towards the end of this period, when the fly population was decreasing rapidly; the fifth series barely reached the requisite age and suffered very slightly.

No explanation of this variation in susceptibility can be advanced at present, as the morphological and physiological changes taking place in the oat plant during growth are apparently unknown.

Under these conditions, the *Arrhenatherum* species were again most heavily infested, although *Hordeum murinum* and *Agrestis myosuroides* followed closely. The difference between the two *Lolium* species is very striking, *L. italicum* being attacked fairly severely while *L. perenne* practically escaped. It is also evident that the fly had a decided preference for certain of these grasses rather than for oats under these particular conditions. The oat plants were in great preponderance numerically but they were more advanced in growth than the grasses, therefore it would appear from these data either that the fly possesses the faculty of selecting suitable plants for oviposition or that, if oviposition is indiscriminate, the larva in some cases fails to reach maturity for reasons connected with plant morphology or physiology. In a previous publication (2) it has been recorded that *Alopecurus myosuroides*, growing wild in the fields among oat plants, may be the more heavily infested, this observation confirming the present data[1]. Records of this nature indicate that grasses, either wild or cultivated, may be of considerable importance in relation to the prevalence of the fly in the field. If the field population is dependent on the grasses rather than the oats, the problem of reducing the extent of the grain infestation is rendered more complex.

REFERENCES.

(1) Cunliffe, N. (1922). *Ann. App. Biol.* ix, No. 2, 165–168.

(2) —— (1921). *Ibid.* viii, Nos. 3 and 4, 170–181.

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[1] If all graminaceous host plants have susceptible periods like the oat, different results may be expected from other age associations of these grasses, because the position of the susceptible period will not necessarily be the same in the cycles of different species.

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FURTHER CONTRIBUTIONS TO THE BIOLOGY OF FRESH WATER FISH. EFFECT OF VARIOUS TRADE WASTES

BY W. RUSHTON, A.R.C.S., D.I.C. (LOND.), M.SC. (ILL.), F.L.S.

IN the April number of the *Annals of Applied Biology*, IX, 1, attention was drawn to some of the effects of trade effluents on the life of the spermatozoa of trout. Further observations have been carried out on young trout using a series of effluents of varied kinds. A certain amount of work has been done on somewhat similar lines in England, the most recent being that of the Joint Board of the Ministry of Transport and Fisheries with regard to the toxic effects of road tar, the Ministries up to the present having issued an interim report but not the full report.

At the commencement of these experiments a series of tests were carried out using the various inorganic and organic substances which are the basis of many trade effluents.

The effect on young trout of most of the alkalies and some salts in dilute solution is to cause a large exudation of mucous from the gills, fins and skin, some being more severe than others, *e.g.* sodium hydrate and sodium carbonate readily cause much mucous to appear at once in stringy masses until such times as the gill covers cannot move on this account. Microscopic examination of the epithelia covering the gills after death show them to be swollen and cleared of granules. Dilute acids, on the other hand, coagulate the mucous as a rule and the epithelial cells are usually shrunk and charged with granules. The effects of distillery effluent were dealt with in the April number, the effects of Flaxwater, Tannery Waste, Woollen Mill, Paper Mill and a gas works are dealt with here.

The effluents vary in effect as would be expected as distillery effluent heavily charged with CO_2 makes the fish lethargic from which condition they recover if removed to fresh water in time. Others have a distinct toxic effect which is fatal almost at once, whilst others bring about a slow lingering death.

FLAX WATER.

Water in which flax has been retted is known to be poisonous to fish. In Ireland damage is done by its careless release from retting pits. Both green and dried flax are retted, a sample of dried flax was used for these experiments. Soft water is stated to be best for the purpose, but London tap-water was used in this case. The flax water was obtained by taking commercial dried flax and steeping it in water at laboratory temperature, the amount used was approximately 300 gms. of flax placed in 100 litres of water. After 24 to 48 hours a vigorous fermentation was set up, much gas rising to the surface. After three days gas production ceased and the liquid became slightly acid, which increased sufficiently to check the further action of the bacteria causing the fermentation. After a week onwards a gelatinous scum began to appear on the surface to a thickness of about an inch, and a strong sulphuretted hydrogen smell prevailed.

Fish were introduced at various stages of the process with the following results:

1. Introduction of yearling trout at the beginning of experiment. No marked effect was shown till after the expiration of 24 hours, when the fish showed signs of becoming sleepy with a tendency to remain in one place refusing to take food, and at the end of two days all the fish originally introduced were dead.

2. Fresh fish introduced at this stage lived for 4–8 hours appearing from the first very lethargic.

3. Two rainbow trout were introduced into the flax water after four days when the fermentation was beginning to subside and the water had become dark brown in colour. The fish were at first very active and made determined efforts to jump out of the tanks, then gradually settled to the bottom breathing rapidly and slowly turned on their backs, with gill covers widely extended. Occasional darting took place and death occurred after about 45 minutes. When dead, the fish had their mouths and gill covers wide open with the gill filaments congested with blood.

4. A quantity of an eight-days' old culture was diluted with an equal volume of water, and, into it introduced two rainbow trout. The effect was similar to the effects with the younger culture but more rapid. One of the fish died within ten minutes, the remaining one was at once removed to fresh running water and recovered from the effects in half an hour. The gills of the dead one were intensely red at the tips of the filaments.

5. The culture was allowed to continue for over two months, during the latter part of which the smell of sulphuretted hydrogen was very apparent. Equal parts of an eight weeks' culture and water were taken, into which were introduced four rainbow trout and one stickleback. As soon as introduced, the fish began darting from side to side keeping near the surface, the stickleback being least affected. All the fish turned on their backs in under five minutes with much aimless darting, and within ten minutes one of the trout was dead. Removing the other three and placing one in running water, another in diluted flax water with a current of water running through, and the third into flax water into which powdered slaked lime had been well mixed and allowed to settle, gradual recovery took place, the last to recover being that where lime had been used.

From the above experiments it would appear that there are three causes which render flax water harmful to fish:

1. Excess of carbon dioxide and a deficiency of oxygen.
2. A rise in acidity.
3. Presence of sulphuretted hydrogen in the water.

Enquiry has shown that damage to fish life in streams occurs when the flax water is liberated at low water and that in times of flood little or no harm appears to follow.

TANNERY WASTES.

The wastes from tanneries are known to be harmful to fish and pollution by them is not uncommon. A few months ago I was allowed to visit a tannery in Scotland where pig skins are prepared for usage in the preparation of hand-bags, purses, etc. and directed to take samples of the four main effluents previous to discharge into the drains.

The effluents were four in number:

1. Lime solution which is used to swell the hide and loosen the hair for easy removal. This effluent contained much lime in suspension and large amounts of organic matter, and gave off a very offensive smell.

2. Bate liquor. The composition of this in the fresh state I was unable to ascertain, but it is used to steep the hides after the lime treatment and neutralises the action of the lime, bringing the hides into a pliable condition. The hides if kept too long in this liquor will putrefy and disintegrate. When cold, it is neutral to phenol phthalein and litmus; on warming, it becomes pink and gives a slight alkaline reaction.

3. Tan liquor is used after bate liquor. This is obtained by steeping bark and nut shells in water. When most of its properties are spent, the

remains are run to waste which at this time gives a strong acid reaction and is heavily charged with foreign matter of inorganic and organic origin.

4. Chrome liquor. Used after the tan liquor. This contains potassium bichromate and sodium hyposulphite in varying amounts. As the various effluents are mixed before turned to waste, varying results would arise as one liquor had a tendency to counteract the other.

The following experiments were done using separate wastes and mixtures, using yearling brown trout in all cases.

Exp. 1. This sample, direct from the tannery, had much lime in suspension which was allowed to settle out. Two and a half litres of the top liquor were decanted off and made up to 100 litres with tap water, giving a dilution of 1–40. Yearling trout introduced, soon became torpid, remaining motionless in one corner of the vessel for 30 minutes, when they gradually lost equilibrium and shortly afterwards died. Repeating this experiment but removing the fish to fresh water on first signs of turning on their side, it was found impossible to get the fish to recover and within an hour they were dead.

Exp. 2. A dilution of 1–1000 was made and fish introduced which, after a period of two hours, appeared to be dead, but on transference to quick running water they recovered in about four hours.

Exp. 3. Bate liquor. A dilution of 1–40 of this liquor seemed to have little effect on the fish beyond a slight increase of respiration accompanied by some sluggishness. Leaving the fish periods of two, four, eight and ten hours and then transferring to fresh water, the fish soon appeared normal.

Exp. 4. Tan liquors. Tests were made with dilutions of 1–40 and 1–1000, and all symptoms were similar to what one gets if solutions of tanning from cutch are used. Cutch is a commercial substance of vegetable origin much used in treating fishing nets and sails. It consists of fragments of a hard, dark-coloured resinous substance and contains about 20 per cent. of tannin.

Two gms. of cutch were extracted with water and made up to 100 litres, the result is a dark brown liquid resembling weak tea. Fish introduced into this, soon show signs of disturbance. The head moves quickly from side to side in quick jerks, they swim wildly and irregularly from side to side for about 15 minutes then settle down in one corner, and for four hours never alter their position unless mechanically disturbed. After four hours the fish were removed to running water and after several hours gradually recovered their normal movements. For three days afterwards whether tan liquor effluent or extract of cutch was used, the fish refused all food. The tannin liquor appeared to be partly swallowed, which would affect the mucous membrane of the oesophagus and stomach in a way which would seriously affect digestion, in addition to having a detrimental effect on the gills. Too long immersion of fish in this liquor proved fatal.

Exp. 5. Chrome liquor. This was a green-coloured effluent and a dilution of 1–4000 was made from the crude waste. Fish introduced were at first very active, moving restlessly about and keeping as near the surface as possible. After 45 minutes the fish had a tendency to swim in a vertical position head upwards with increased rate of respiration. Bubbles of gas occasionally escaped from the mouth. The fish were left for two hours in this solution after which they were removed to fresh water and for a time appeared normal, but within 24 hours the fish appeared out of sorts and died within four days. After death the mouths were closed, gills brownish-pink in colour and the epithelia covering the gills disintegrated and easily stripped off. Dissection showed the lining of the gut inflamed.

Exp. 6. A mixture of the four liquors, 100 c.c. of each being taken and made up to 100 litres. The mixture gave an alkaline reaction and contained a little suspended matter, brown in colour and gave off an offensive smell. Yearling trout placed in this mixture showed active disturbances at once and within 15 minutes were overcome beyond recovery. Repeating the experiment but removing to fresh water at the first signs of trouble, it was found impossible to recover the fish. This experiment was tried to test a theory prevalent in certain circles that while tannery effluents are harmful apart, a mixture of the four gives a harmless effluent.

EFFLUENT FROM WOOLLEN MILL.

Only diluted mixed samples of effluents from a woollen mill were available, and the constituents of these may vary from time to time due to the source of the waste products.

Three main sources of waste are possible:

1. From the wool scouring vats.
2. From the mordanting vats.
3. From the dye vats.

As a rule the effluent contains all three in different proportions. The method of wool scouring is well known and consists of washing the raw wool in a hot alkaline liquid which removes large quantities of natural fats and dirt, when the process is finished the partly spent wool scouring liquor is usually poured into the neighbouring stream and consists of a dark soapy liquid which discolours the water and is strongly alkaline. The effect on fish is very marked and is very similar to what one gets if a strong freshly-made solution of sodium carbonate is used. The alkali stimulates the activities of the mucous cells around the gills and causes a heavy discharge of mucous which forms stringy masses at the edges of the fins and gill covers, finally causing the gills to be put out of action by its accumulation. Microscopic examination of the epithelia covering the gill filaments, shows the individual cells to be swollen up and cleared of granules which are the forerunners of mucous.

Fish introduced into the crude effluent at once show signs of irritation, the fish only being seen when near the surface. The eyes seem much affected, making unusual movements in a forward direction, the stringy mucous appears on the fins, etc. and although it takes many hours to kill the fish, it does ultimately result in death if they are left too long. If after two hours the fish are removed to running water, they recover in the course of a few hours.

It would appear that fish are able to withstand a sudden flush of wool scouring liquid more easily than many effluents, especially if the effluent is free from ammonia.

From mordanting vats. The mordants used at this particular woollen mill are alum and potassium dichromate, both of which alone are deadly to fish. The amount of mordant in the effluent is not usually very much, as most of it is recovered on account of its high price. It is usually coloured to varying extents with dye liquor.

Its effect on fish is similar to that of chrome liquor from a tannery. In no case was it possible to recover fish after an immersion of more than one hour if an amount of more than 1–40,000 of mordant was present.

EFFLUENT FROM A GAS WORKS.

The effluents from a gas works depend on the part of the plant from which they are drawn, but the particular effluent under consideration here was from the overflow of the ammonia collecting tanks which, as analysis shows, contained parts per 100,000:

Dissolved solids ...	68·00	Albuminoid ammonia	1·64
Suspended matter ...	35·00	Tar acids	23·84
Chlorine	21·30	Tar bases	6·00
Free ammonia ...	15·00	Dissolved oxygen ...	0·30

The sample was obtained from a river just where the effluent had entered and fish were killed by its action. The effect on trout and grayling was marked by the gills being intensely red and swollen, the blood vessels of the gills gorged with blood and the covering epithelia swollen and free from granules. An excessive amount of mucous surrounded the gills. The effects were also shown in the gut. The oesophagus in all cases was locally inflamed and the walls of the stomach and intestine inflamed throughout. No parasites of any description were present in the gut. *Heart.* All vessels gorged with dark coloured blood.

The appearance throughout was similar to what one gets if dilute solutions of free ammonia are used on fish. The analysis shows 15·00 parts per 100,000 of free ammonia. Experiments using 3·00 parts per 100,000 have been found to kill trout in 20 minutes. Mason found 5–100,000 to kill in 47 minutes; Clark and Adams found 1–100,000 to kill in under an hour.

With tar acids the analysis shows 23·84 parts per 100,000. Experiments using 15–100,000 killed trout in 10 minutes; Rufter and Baker, using 33–100,000, killed trout in 5 minutes; Mason, using 1·5–100,000, killed trout in 15 minutes.

Gas water from top of the wells are similar to the sample under consideration. 1–100,000 killed perch in a few hours. Waste water from gas works, 0·5 per cent., kills trout, according to Knight, in 10–15 minutes. Analysis shows oxygen in solution to be under 50 per cent. normal, which is near the danger line for trout.

EFFLUENTS FROM PAPER MILLS.

The effluent from two paper mills has been investigated, one in Scotland, where the effluent enters a well-known salmon stream about 20 miles from the mouth, and another in the south of England on a tributary of the Thames. In regard to the northern one the effluent lodges in pools and has the effect of killing salmon which may find their way into it in times of low water, in this case as many as 25 fully-grown salmon being picked up within a few weeks.

Examination of one of these fish immediately after death showed the gill filaments very red and swollen, a slight excess of mucous in the mouth and on the tongue. Internally the liver was very dark on the outside and mottled in section. Spleen almost black, oesophagus, stomach and intestine intensely inflamed. Microscopic appearance of the gill epithelia showed the cells swollen.

A crude sample of the effluent was used on salmon fry, four of which were placed in a litre of effluent side by side with four in tap water, with the result that of the ones subjected to the effluent, two died within two hours and the other two within eight hours. When dead, the mouth and gill covers were open to their widest extent, gill filaments greyish and swollen, covered with an excess of mucous, but not to the same extent as with a strong alkali. The blood vessels of the gut were much congested.

A second sample, more alkaline than the first, killed fry in three hours, strings of mucous appearing as a halo round the fins and body of the fish with the gill filaments intensely red and swollen.

In the case of the southern stream it has been possible to study the effects of the effluent on the stream itself, where its results can be seen. In this case from the mill are poured out two effluents, one from the washing machines and the other from a source which receives much of the waste oil. Both of these effluents are poured into a canal which runs somewhat parallel with the stream from which it overflows at various points into the stream.

The effect on the canal is that, below the effluent outflows, no fish of any kind exist and the vegetation is remarkably altered, many of the plants found above the mills being missing for some distance below. Examination of the various overflows from the canal to the river shows rich growths of fungi on the bed of the stream and the water is very cloudy. The effect on the stream is, that for some distance down, few, if any, trout are present, but above, they are plentiful and about four miles below they are plentiful. Although trout have been introduced from time to time in this polluted area they will not stay, but migrate up-stream or fall back down-stream.

A series of chemical analyses of the water from the river and canal above effluent outflow, crude effluents from the paper mill, diluted canal water flowing into main stream, and stream below effluent outflows give the following results which may vary from time to time depending on the rate at which effluent is entering canal.

The figures tend to show that the area affected by the effluent is sufficient to drive the fish away, and within the last few years has not only removed them but completely altered the nature of the stream in regard to vegetation and natural fauna, due to the constituents of the paper mill's effluents.

In many cases where chemical analysis has not yielded any result which would indicate the nature of the toxic substance, fish have been known to be affected, experience leads one to the conclusion that biological as well as chemical examination is important in investigating the deleterious effects of trade wastes on streams. The question of these

Analyses of Stream, Canal and Paper Mill Effluents.

	Stream above Paper Mill	Stream a mile below Paper Mill	Stream two miles below Paper Mill	Canal above Paper Mill	Canal 500 yds. below Paper Mill	Canal ¾ mile below Paper Mill	Canal two miles below Paper Mill	Crude eff. 1.	Crude eff. 2.
Physical characters	Clear	Slightly cloudy	Slightly cloudy	Clear	Cloudy	Cloudy	Cloudy	Opaque	Opaque
Colour in 2 ft. col.	Pale yellow	Cloudy	—	—	—	—	—	—	—
Reaction	Slightly alkaline	Neutral	Slightly alkaline	Slightly alkaline	Faintly acid	Faintly acid	Faintly acid	Acid	Acid
	Analyses in parts per 100,000, except oxygen in solution, which is in mg. per litre.								
Solids in solution	20·00	22·00	15·00	22·00	40·00	30·00	25·00	120·00	85·00
Chlorine as chlorides ...	1·1	1·5	1·5	1·5	1·6	2·0	1·8	4·8	2·9
Ammonia free and saline ...	0·01	0·019	0·010	0·008	0·100	0·01	0·009	0·16	0·16
Ammonia albuminoid ...	0·014	0·026	0·020	0·012	0·86	0·02	0·018	0·22	0·20
Nitrates	0·2	0·25	0·10	0·20	0·3	0·18	0·150	0·54	0·38
Nitrites	nil	Trace	nil	nil	Trace	Trace	nil	Trace	Trace
Oxygen absorbed from potassium permanganate in 4 hrs. at 27° C.	0·03	0·09	0·082	0·03	0·10	0·52	0·45	2·00	1·6
Oxygen in solution, temp. 10° C.	9 mg.	8·7	7·5	8·5	8·2	4·5	4·5	1·0	2·0
Acidity as sulphuric acid ...	Slightly alkaline	Neutral	Neutral	Slightly alkaline	Faintly acid	Faintly acid	—	5·8	2·2

trade wastes is becoming of vast importance to the biologist, as so many of our streams are being gradually changed in character. They have also their economic bearing, indicating that many useful substances are being allowed to run to waste.

CONCLUSIONS.

1. Substances may be present in a stream in such small quantities as to escape chemical detection but may be deleterious to fish life.
2. The character of a stream may be totally altered, due to the effects of trade wastes being poured into it.

REFERENCES.

Stream Pollution Studies. State of New York Conservation Commission. 1922.
Interim Report for Pollution Commission. Ministry of Fisheries. 1922.

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EFFECT OF WEIGHT OF SEED UPON THE RESULTING CROP

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(With 5 Text-figures.)

THE question of the influence of the quality of the seed sown upon the resulting crop is one of paramount importance to the agriculturist, and has received a good deal of attention in the form of field trials. The results obtained are not very consistent, though the general trend is in favour of the well developed, heavier seeds. The reason for this apparent discrepancy may be due to the fact that the problem has really two aspects, which have not always been recognised and separated. In one aspect the relative weight of the seeds is estimated by the weight per bushel, in which case all the small, shrivelled and cracked seed that is screened out from a large bulk is included with the light seeds, giving a smaller weight per bushel than the remaining large seeds which are free from rubbish. In the other aspect the rubbish is not included, the comparison being made between good seed in each case, the difference being only in the size and weight of the individual seeds. This does not necessarily entail that the smaller seeds should give a less weight per bushel, though doubtless this does frequently happen. Then again the question arises as to whether the comparison is to be made with the crops obtained by sowing on similar areas

(1) equal quantities of seed by *weight*, the lighter seeds giving a larger number of plants per acre;

(2) equal quantities of seed by *volume*, in which case again the lighter seeds, being usually smaller, give more plants per acre than the heavier seeds;

(3) equal *numbers* of seeds, when the comparison becomes a true one as regards the productivity of individual plants.

All three methods have been used by investigators.

Zavitz (19) carried out experiments for several years with various field crops, testing harvests resulting from the use of large, medium and small seeds, equal numbers of seeds being sown in each case on equal areas. Averaging the results per acre for the whole period he found a marked superiority in favour of the large grains, amounting to as much as 19·1 per cent. for grain crops, 40·3 per cent. for rape, and 60·1 per cent. for root crops. Much variation occurred between the individual crops, as among the cereals oats gave the greatest average increase of 33 per cent., and spring wheat the least increase of 16·5 per cent. over that obtained with small seeds. Less variation appeared among the root crops, all those tested showing marked improvement from the use of large seed. The results are sufficiently striking to merit reproduction.

Table I. *Zavitz's results with Field Crops.*

Equal numbers sown with all sizes of seed on equal areas.

	Crop	No. of years' tests averaged	Yield per acre		
			Large seed	Medium seed	Small seed
			Bush.	Bush	Bush.
Grain	Oats	7	62·0	54·1	46·6
	Barley	6	53·8	—	50·4
	Spring wheat	8	21·7	—	18·0
	Winter wheat	6	46·9	—	40·4
	Field peas	6	28·1	—	23·0
			Tons	Tons	Tons
Roots	Mangels	5	33·2	29·6	21·5
	Sugar beets	5	22·9	21·9	14·3
	Swede turnips	5	17·1	15·2	8·7
	Fall turnips	4	25·4	21·7	16·2
	Field carrots	5	24·5	22·2	16·2
Rape	Rape	5	17·4	15·0	12·4

In this connection wheat has been repeatedly tested, especially in the United States. Thirty years ago Georgeson and his collaborators (8, 9) arrived at the conclusion that there was no doubt of the advantage of sowing seed of heavy weight per bushel, their average results for three years' trials with wheat showing an increase of two bushels per acre of grain, with a possible slight increase in straw production. The light grade seeds are light because they contain a percentage of small and shrivelled grains of less vitality than plump grains, and these may fail to germinate or may produce weak plants. Under very favourable conditions, however, a thicker strand may be obtained from light seed owing to the larger number of seeds sown per bushel, and the difference from the crop obtained from heavy seed may thereby be diminished. For instance, in 1896, Zimmerman wheat of rather poor quality was sown on rich soil (10), and a wet season caused a heavy growth and large yield, which was practically the same from both light and heavy seeds. Hays (11) and his fellow workers emphasised the distinction to be drawn between size and weight of seed in estimating crop results. They practised a system of continuous selection of seed, not merely a yearly selection from the bin as other workers had often done. The actual size of seed seems to be of less importance, as the largest kernels gave poorer yielding varieties than the smallest kernels, but heavy hard kernels produced varieties yielding nearly two bushels per acre more than the largest kernels, the experiment therefore emphasising the importance of selecting seed wheat of high quality and heavy weight. More recently Wheeler and Balz (18) have again obtained an increase of over two bushels per acre in favour of heavy seed in wheat, equal volumes of differing weights per bushel being sown. The heavier seed came up the earlier with a stronger and more vigorous plant, and it stooled out the quicker. Later on the conditions were very favourable for a good grain crop and the plants from the lighter seeds made up some of the early differences, but even so in seven trials the heavier seed gave the bigger crop in every case but one. Occasionally the light seed gave heavier straw but lighter grain, due to the crop from the lighter seed being later and not filling out so well as that from better seed. Other things being equal, the earlier wheat is, the better it fills in an average season. Desprez (5) also advocates sowing large heavy seed wheat, at the same rate by weight per unit area as would be necessary with small light seed. He quotes experiments made at the Station agricole de Cappelle with five varieties of wheat, in which equal areas were seeded with similar weights of large and small grains. In every case a heavier yield of grain was obtained from the larger seed, ranging from 4 to 23 per cent. increase according to the variety, and with three varieties the weight per hectolitre was also heavier, in one instance that from heavy seed being 81·6 kilos. and from light seed 78·4; the other two varieties showed no difference. The yield of straw was sometimes to the advantage of the heavy seed and sometimes of the light, again according to variety.

Bolley (3) conducted controlled tests with a definite number of seeds of known weight. Plump seeds of Scotch Fife were used, 800 weighing 23½ gms. and 800 weighing 12½ gms. being sown one and a half inches apart in rows. The total weight of straw and grain was over 10 per cent. in favour of the heavy seed, and the weight of the heads alone over 12 per cent. in the same direction.

With wheat, therefore, it seems evident that under normal or unfavourable conditions of growth the use of well filled, heavy seed results in a definite increase of crop, frequently about two bushels per acre, but that in seasons very suitable to the growth of cereals this benefit is not always obtained.

Voelcker (16), on the other hand, carried out pot cultures at Woburn to compare the crops obtained from sowing "head" and "tail" corn of wheat and barley, and found no advantage from the use of the heavier seed. This may have been due to the fact that the plants were grown under very advantageous conditions, thus bearing out the general conclusion previously quoted, or possibly a certain shortage of plant food existed in the pots, acting as a limiting factor to growth and tending to level up results in some cases, as no record is given of the application of fertilisers to the soil used.

Zavitz's results with oats, already quoted, confirm the earlier work of Boss (4), who found a decided advantage on the side of heavy seed, far more marked than with wheat. He further stated that his increase of nine and a half bushels per acre, though heavy, was not so great as usual, as under good growing conditions light seeds do better than when conditions are poor, exactly as in the case of wheat.

With barley again, Soule and Vanatter (15) found a constant advantage on the side of heavy seeds. In this case a definite number of seeds were sown per plot, and the large and small seeds were selected for each plot from heads of various sizes, three varieties being tested. Field experiments on the light sandy land at Woburn (17), however, showed no benefit from the use of heavy seeds, rather better crops being obtained from the "tail" than the "head" corn. With maize Georgeson and his fellow workers (7) failed to obtain any advantage from the use of large seed.

Among the leguminous crops, Lehmann (13) with peas, and Fruwirth (6) with lucerne and sainfoin have obtained better crops from the use of heavier seed, though with perennial plants the initial advantage does not persist, but decreases with increasing age of the plant. In experiments on the growth and variability of Helianthus, Reed (14) concluded that plants which were small at maturity were generally small from the beginning, and those which were large at maturity had a well marked superiority from the start.

Illustrations similar to the above might be multiplied[1], but sufficient evidence has been put forward to indicate that in field practice a decided advantage is usually to be obtained by the use of heavy well-filled seed, though under particularly favourable growing conditions the advantage may be decreased, or even, as in the case of wheat, nullified.

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[1] Many further references are given by Kidd and West (12).

Two or three years before the publication of Kidd and West's papers on Physiological Predetermination (12) some hitherto unpublished experiments were made at Rothamsted approaching the question of the effect of seed weight from a rather different standpoint. In field tests like those hitherto described, all the plants, whether from light or heavy seeds, are in competition with one another for food, water and light, and also they are subject to bird and insect attacks, which have much effect upon the resulting crop. Furthermore, earlier experiments had been carried out with numbers of selected heavy and light seeds which were bulked together before being weighed, so that little or no information is available as to the progress of events with individual plants grown from seeds of various weights. Experiments were therefore carried out in water cultures with peas and barley, in which the competitive factors were eliminated as far as possible in order that the results could be more closely correlated with the initial weights of the seeds.

A. SEEDS GROUPED BETWEEN KNOWN LIMITS OF WEIGHT.

The seeds were weighed into groups, the difference between the heaviest and lightest in each group being ·01 gm. for barley, and 0·5 gm. for peas. Pedigree strains of seed were used for barley (Pedigree Plumage) and Sutton's Harbinger[1] for peas, and all were selected from one sample in each case. The seeds were germinated in clean sawdust, and as soon as the seedlings were large enough to handle they were transferred singly to 600 c.c. bottles of nutrient solution[2]. Ten plants of each size of seed were reckoned as a unit.

PEAS.

Seeds sown Oct. 16th and put in solutions Oct. 25th, 1915.

Harvested Jan. 25th and Feb. 22nd, 1916.

Two parallel sets were grown and were harvested after 92 and 120 days respectively. The food solution was not changed throughout the experiment, so that all the growth was made at the expense of the initial supply in the nutrient solution, the stock stored up in the seed, and the carbon assimilated from the air. The peas grew from Oct. 25th through

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[1] I am indebted to Mr Martin Sutton for the gift of the pea seeds used throughout these experiments.

[2]

KNO_3	1·0 gm.	Distilled water to make up one litre.
$MgSO_4$	·5 "	
NaCl	·5 "	
KH_2PO_4	·5 "	
$CaSO_4$	·5 "	
$FeCl_3$	·04 "	

the winter, and when put into the solutions very little difference was observable between the roots or shoots from large and small seeds. Eighteen days later a regular gradation in size was evident, this being obvious in the roots as well as in the shoots. At this early date there could be no question of a deficiency of food and the greater growth from the heavier seeds would seem to indicate a capacity for making better use of the available food supply at an early stage, as the extra food stored up in the seed is probably more readily available than that resulting from photosynthesis in the young plants. A slight initial advantage obtained in this way would have a considerable effect upon the after growth by the working of the compound interest law, as has been pointed out by V. H. Blackman (1). After 13 weeks' growth the dry weights of the plants showed the same steady gradation as was observed by inspection of the growing plants, the proportionate rise being similar in the shoots and the roots (Table II and Fig. 1). It is often stated that this increase in weight is due to the extra food supplied in the seed. Although this statement is true in effect it is rather misleading, because the extra dry matter formed by the plants from the heavier seeds is in excess of the extra supply in the seeds, the difference in weight of the plants from the lightest and heaviest seeds being in this case twice the difference in the weight of the seeds. A truer view of the matter is that the larger store in the seed enables the plant to make a more rapid initial growth which gives it an advantage that persists in after life.

A month later, after 17 weeks' growth in the original nutrient solution, the difference between the plants from the light and heavy seeds was still very marked, though the discrepancy was not quite so large as at the earlier date (Table II and Fig. 1). Reckoning the weight of the plants from the lightest seeds as 100, after 13 weeks the ratio of the weight of the heaviest to the lightest was 225 : 100, and after 17 weeks only 167 : 100. This probably indicates that the larger plants had almost exhausted their food supply and were growing less rapidly, while the smaller plants had more available food left and were utilising it, thus decreasing the comparative advantage gained by the larger plants. The actual difference between the weights of the large and small plants increased with age, becoming constantly greater than the difference between the original weights of the seeds, thus affording additional proof of the hypothesis that the heavier seeds give the plants a certain initial advantage which makes itself felt throughout life. Growth during the first 92 days was very slow, being in the depth of winter when the light intensity was much depressed, and when harvested the plants from even

Table II.

Peas. Sown Oct. 16th, 1915. Averages of ten plants.

	Grown for 92 days			Grown for 120 days		
Weight of seed gm.	Total dry weight gm.	Shoot/root	Effic. index	Total dry weight gm.	Shoot/root	Effic. index
Under ·1 gm. (1 plant only)	[·174	4·26	·9148]	[·398	6·22	1·391]
·1 –·15	·344 ± ·005	4·23 ± ·129	1·10	·955 ± ·032	4·85 ± ·15	1·695
·15–·2	·387 ± ·010	4·49 ± ·166	·8628	·990 ± ·029	5·01 ± ·13	1·444
·2 –·25	·470 ± ·012	3·85 ± ·106	·8008	1·144 ± ·027	4·90 ± ·08	1·355
·25–·3	·543 ± ·015	4·21 ± ·111	·7395	1·254 ± ·038	4·82 ± ·12	1·273
·3 –·35	·693 ± ·014	4·28 ± ·174	823	1·464 ± ·022	4·03 ± ·14	1·254
·35–·4	·776 ± ·012	4·05 ± ·084	·7904	1·596 ± ·030	3·87 ± ·06	1·207

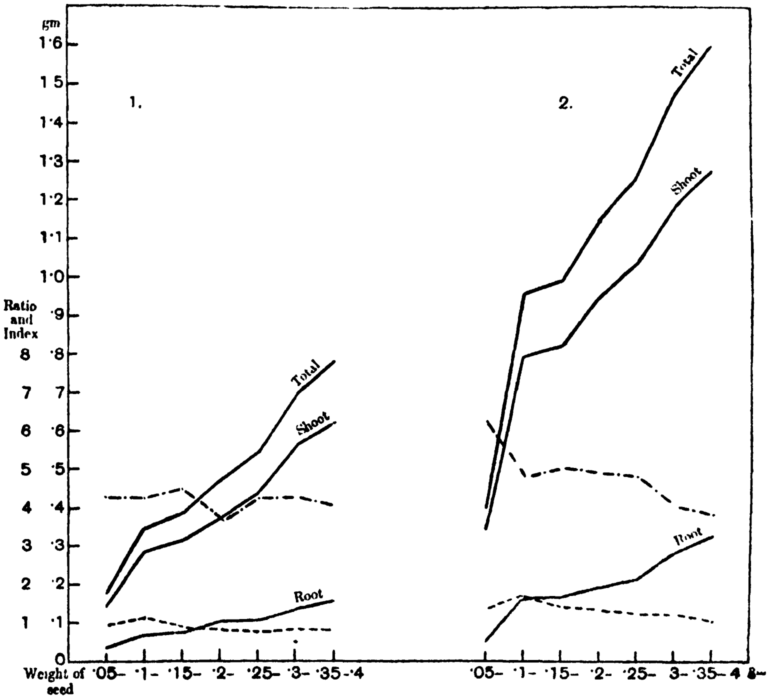

Fig. 1. Harbinger Peas grown from groups of seeds increasing in weight by ·05 gm. in each group. Averages of 10 plants. 1. Oct. 16th, 1915–Jan. 25th, 1916 (92 days). 2. Oct. 16th, 1915–Feb. 22nd, 1916 (120 days). Solutions not changed.

———— Dry weight. —•—•— Shoot/root ratio. - - - - - Efficiency index.

the heaviest seeds could not have made very considerable inroads on their supply of food. Nevertheless, the dry weight of the largest plants differed from that of the smallest by four times the difference in seed weight. Under these circumstances there was no significant difference in the rate of growth of the shoot and root, demonstrated by the shoot/root ratio. The efficiency index, too, was very similar in all cases, though it was possibly a trifle higher with the very light seeds. After longer growth, under improving conditions of illumination, the plants from the very heavy seeds grew so much more than the others that they depleted the nutrient solutions to a greater extent and in this case the roots from the heaviest seeds continued to grow more rapidly than the shoots, thus depressing the shoot/root ratio. The efficiency index (excluding that of the single plant from a seed below ·1 gm.) gradually fell as the weight of the seed decreased. The possible correlation of this fall in the shoot/root ratio and the efficiency index with limited food supply could not be worked out at this stage, but will be discussed later in connection with further data that have since become available (see pp. 236–8).

BARLEY.

In the first experiments with peas a definite limiting factor was at work in that a restricted amount of food was supplied for the whole period of growth. The action of this limiting factor was investigated in barley by the observation of plants which received a restricted and an abundant supply of food, grown for 67 and 162 days respectively. In the former case the results resembled those with peas under similar conditions, *i.e.* the heavier the seed, the greater the ultimate growth. With barley the difference was more strongly marked, as after only nine weeks' growth the difference in weight of the largest and smallest plants was nine times the difference in the initial weight of the seeds (Table III and Fig. 2). With an abundant food supply (*i.e.* when the food solution was constantly renewed) and a longer period of growth the barley reached maturity, forming ears and ripening seed before harvesting. In the earlier stages of growth steady gradation in size was apparent according to the weight of the seed, but gradually this became less visible, though the difference was still marked if the plants from the lightest seeds were placed alongside those from the heaviest. By harvest time little difference could be observed, as the plants were so large that minor variations were obscured. The dry weights, however, showed that some difference still existed, as the heaviest plants were still obtained from the heaviest seeds. The roots had ceased to show any difference, and the increased weight was manifest only in the shoots, but the mean difference between the lightest and heaviest plants was over 60 times the difference between the initial weights of the seed. The relative growth of shoots and roots, as

Table III.

Barley. Sown Feb. 22nd, 1916. *Average of ten plants.*

	Solutions never changed. Grown for 67 days			Solutions frequently changed. Grown for 162 days		
Weight of seed gm.	Total dry weight gm.	Shoot/root	Effic. index	Total dry weight gm.	Shoot/root	Effic. index
·01–·02 (1 plant only)	—	—	—	[27·50	6·65	4·637]
·02–·03	·821±·056	1·949±·084	5·213	29·26±·71	4·895±·043	4·327
·03–·04	·848±·038	1·790±·046	4·758	28·00±·31	5·451±·192	4·127
·04–·05	1·029±·045	2·036±·088	4·671	28·45±·24	5·107±·210	3·981
·05–·06	1·190±·055	1·997±·072	4·588	29·94±·60	5·397±·116	3·889
·06–·07	1·270±·037	2·597±·104	4·436	30·75±·60	6·151±·263	3·803

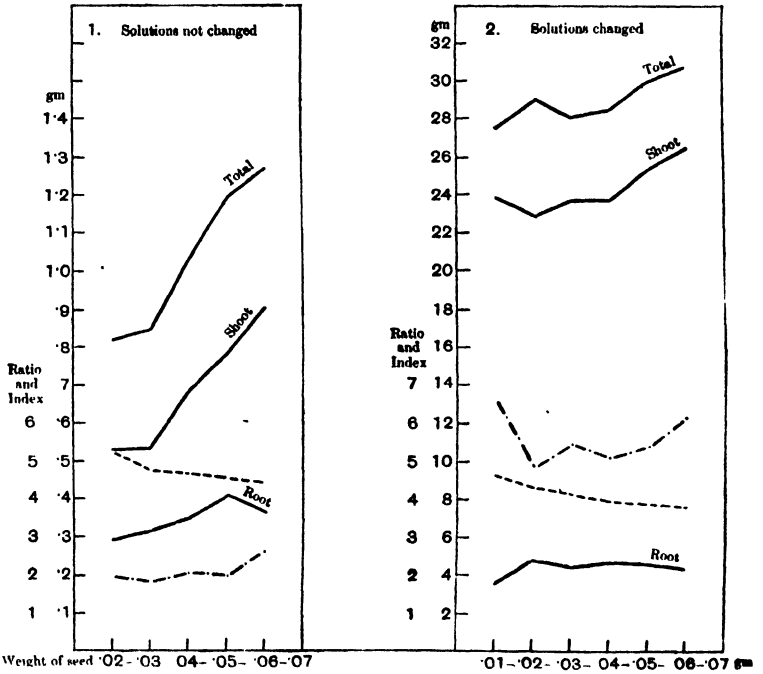

Fig. 2. Barley grown from groups of seeds increasing in weight by ·01 gm. in each group. Averages of 10 plants. 1. Feb. 22nd–April 29th, 1916 (67 days). 2. Feb. 22nd–Aug. 2nd, 1916 (162 days).
———— Dry weight. —•—•— Shoot/root ratio. – – – – – Efficiency index.

shown by the shoot/root ratio, was little affected except in plants from the heaviest seeds, in which it tended to rise, the actual value being influenced by the scarcity or abundance of food supplied. The efficiency index under both sets of conditions fell slowly with increasing weight of seed (see p. 238). The change in the apparent gradation in the size of plants with age was also illustrated by the quantity of nitrate used up by the plants at different periods.

Table IV.

Potassium nitrate utilised by barley plants.

	Weight of seed gm.	→	·01–·02	·02–·03	·03–·04	·04–·05	·05–·06	·06–·07
	Nitrate used in — days	After-days previous growth		Solutions frequently renewed				
			gm.	gm.	gm.	gm.	gm.	gm.
Ap. 18	7	35	·335	·425	·51	·58	·629	·717
May 15	6	63	·885	·96	·945	·977	·963	·965
May 21	5	69	·87	·905	·852	·915	·96	·963
May 26	6	74	·73	·848	·865	·945	·882	·907
				Solutions never renewed				
Ap. 19	43	—	—	·545	·595	·695	·75	·75
May 1	55	—	—	·712	·725	·783	·83	·847

The solutions were changed weekly or oftener, and analyses were frequently made of the nitrate remaining in them at the time of renewal, 1 gm. per litre being supplied on each occasion. For the first few weeks the nitrate used up by the smaller plants was considerably less than that by the larger, a gradation occurring according to the size, twice as much nitrate being absorbed by the largest plants as by the smallest. As time went on the difference became very much less, all plants utilising the nitrate freely, though those from the lightest seeds absorbed appreciably less to the end of the experiment. When the nutrient solutions were not renewed, thus limiting the available food supply, the nitrate was never so thoroughly exhausted from the solution even by the plants from the heaviest seeds, but these still utilised considerably more than those from the lighter ones (Table IV).

In the experiments considered, where the food solution was not changed, the length of the period of growth was determined by the food supply, as it was necessary to harvest the crops before starvation deteriorated or killed the plants. Consequently, the peas formed but few pods, if any, and the barley was cut before the formation of ears, and under these circumstances the effect of the size of the seed upon growth was strongly marked, especially in peas. The weight of seed may therefore be of special importance in the case of crops that are normally cut before reaching maturity, being grown for their leaves rather than for fruit and seed.

B. SEEDS OF KNOWN INDIVIDUAL WEIGHT.

PEAS.

The results obtained with the grouped seeds raised the question of how the plants from individual seeds of known weight would behave in respect to total growth and rate of increase. A number of Sutton's Harbinger peas were therefore accurately weighed and experiments were carried through in the early autumn and in the spring, at times when good normal growth was to be expected. The seeds were germinated in damp sawdust, being kept separate by pieces of cardboard recording the initial weights of each seed. As soon as large enough the seedlings were transferred to water cultures and divided into three sets, each set running up the scale from the lowest to the highest weights. In two sets the solutions were constantly renewed, but in the third no fresh nutrients were supplied, distilled water only being added as necessary. When the unchanged set showed signs of suffering from lack of food the plants were harvested, together with one set growing in the changed solutions, the others being allowed to grow on for some time longer before cutting. Two experiments were carried out, but the results were generally so similar that they may be considered together, the figures given being those of the autumn test:

Seeds sown Aug. 31st, ranging in weight from ·095–·421 gm.
Put in solutions Sept. 8th.
Harvested Nov. 16th (solutions changed and unchanged).
,, Dec. 8th (solutions changed).

When put into solutions the plants from the heavier seeds were distinctly ahead of those from the lighter both in shoots and roots. At an early stage the roots of the larger plants developed strong sturdy rootlets, which were thickly placed down the main roots and were closely studded with laterals in their turn. Those from the lighter seeds developed much thinner rootlets which were spaced further apart on the main root and were not so well supplied with laterals themselves. These phenomena occurred as a *general tendency* and could not, at this stage at least, be interpreted as representing a fixed rule in the gradation of plants from light or heavy seeds. In the earlier stages of growth, the leaves from heavy seeds were bigger than those from light ones. At all the harvests the appearance resembled that obtained in the earlier experiments, the general gradation of large to small plants from heavy to light seeds being clearly evident. Each plant was harvested individually, and the total dry weight and the efficiency index of each obtained. The results are graphed in

Figs. 3 and 4, but for considerations of space the actual figures are not listed. In this connection attention should be drawn to the fact that in all these experiments with weighed seeds the efficiency index is perforce rather too low. It was naturally impossible to find out the initial dry weight of each seedling when put into the solution, as that would have entailed its destruction, so it was necessary to use the original weight of the seed for W_0 in calculating the efficiency index. Other experiments(2) have shown that at this stage the seed has lost a good deal of its original weight by respiration and that this has not yet been made up by photosynthesis, so the initial weight (W_0) used is rather too high and tends to reduce the value finally obtained for the index.

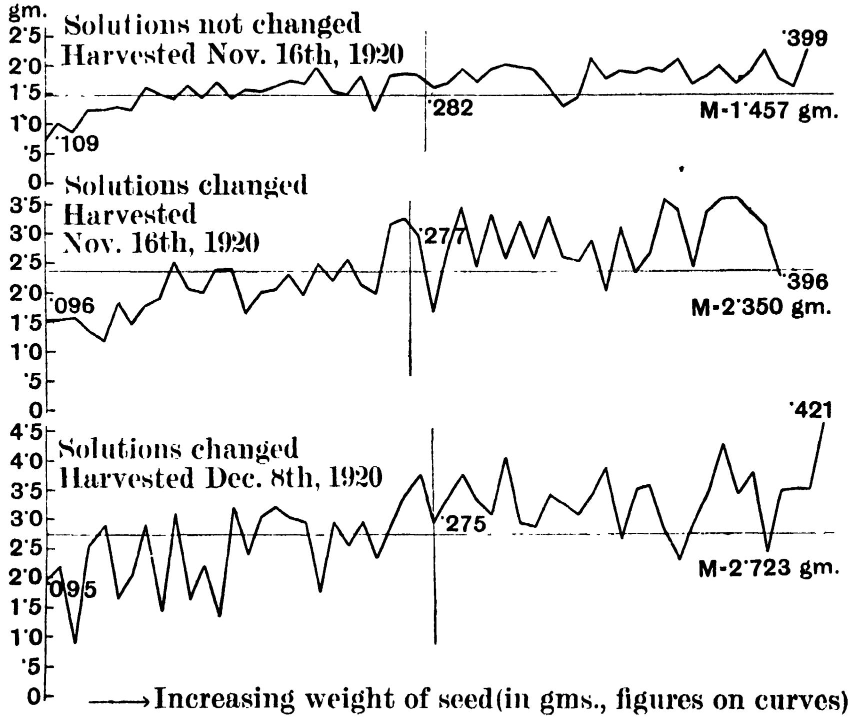

Fig. 3. Dry weights of three series of Harbinger Peas grown from seeds of known individual weight, sown on Aug. 31st, 1920. The line *M* indicates the mean of the highest and lowest dry weights in each series. The vertical lines divide the series into two equal parts.

In the presence of a limited supply of nutrients flowering was retarded with all the plants and few pods were produced, those that did form being small and undeveloped. With plenty of food all flowered freely, but on the smaller plants the pods remained small and poorly developed, whereas on the larger plants they filled out well. The gradation between the plants from light to heavy seeds was very marked both in shoots and roots, especially where unlimited food was available, and this was borne out by the dry weights at harvest. These showed considerable variation in plants from seeds of much the same weight, but the trend of the curve was always upwards. This is well shown if the mean dry weight of the lightest and heaviest plant in any set be taken as a datum line and the plants from the lighter and heavier seeds compared with it. For instance, in one case (see Fig. 3, 2nd curve), where mean dry weight was 2·350 gms.,

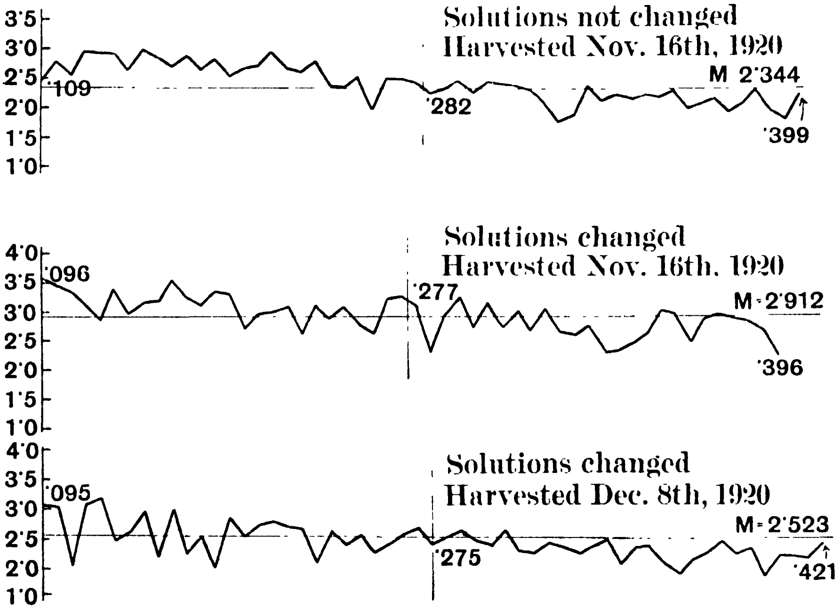

Fig. 4. Efficiency indices per cent. per day of three series of Harbinger Peas grown from seeds of known individual weight sown on Aug. 31st, 1920. The line *M* is drawn for purposes of comparison at the mean of the highest and lowest efficiency indices in each case. The vertical lines divide the series into two equal parts.

Among plants from 26 lightest seeds (·096–·268 gm.)

7 dry weights were above mean, and 19 below.

Among plants from 26 heaviest seeds (·277–·396 gm.)

22 dry weights were above mean, and 4 below.

Similar figures could be shown for every set.

If, however, the peas were grown on for some time and allowed to approach maturity, it was found that above certain weights the advantage of the heavier seed became less, though it was still very marked among the plants from the lighter seeds. This is best seen by grouping the plants into quartiles and striking the mean dry weight in each; this rises steadily and rapidly with the weight of seed except in the last quartile, in which comparatively little advantage appears on the side of the heaviest seeds (Fig. 5 and Table V). This indicates that after a certain stage the initial dry weight of the seed ceases to have any influence upon the resulting crop, at least so far as plants derived from reasonably heavy seeds are concerned. With an annual crop such as peas, natural death occurs soon after this is manifest, while the advantage of heavy seeds over light is still very marked, but it is quite conceivable that with perennial plants the process might continue down the scale until ultimately, after various periods of time, the plants from light seeds were as good as those from heavy ones. In any case with short lived annual plants like peas and barley, there is an obvious advantage to be gained from using heavy well-developed seeds. An explanation of the change in the relative advantage of the seed with age is evident when the efficiency indices are examined (see Figs. 1, 2, 5). In every case, whether with limited or abundant food supply, or whether with a short or long period of growth, the rate of increase of dry weight per cent. per day with peas and barley slowly decreases as the initial weight of the seed increases. The initial capital, represented by the seed, is so much greater in the case of the heavy seeds that even with this lower rate of increase the total weight reached at compound interest for a long period of time rises much above that attained by lighter seeds with a rather higher efficiency index. Eventually, however, the difference in the rate of increase gradually begins to tell, and plants from seeds that were not much below the heaviest in weight pull up into line and become as heavy and well-developed as those that hitherto were their superiors. If healthy growth continues without death supervening, the process goes on, and still lighter seeds produce plants equal to the best. It is, of course, only hypothesis at this stage to assume that this would go on indefinitely until all initial advantage from weight of seed were wiped out, but if it be true, it affords a reasonable explanation of the results of Fruwirth, supporting his contention that with perennial plants the initial advantage of heavy seeds disappears with age. The lower rate of increase with the heavier seed cannot be attributed to any failure in the food supply, as when plenty of food was given the solutions were renewed so frequently that comparatively little of the nitrogen was removed (usually not more than 20 per cent.) before a full supply was again offered. It would rather seem to be due to a gradually increasing inability of the embryonic shoot and

Table V.

Peas sown Aug. 31st, 1920.

Results from about 56 plants in each case, grouped into quartiles of 13 or 14 plants and averaged up.

Mean weight of seed gm.	Total dry weight gm.	Shoot/root	Efficiency index
	Solutions not changed. Grown for 77 days		
·154	1·295±·051	5·24±·11	2·75
·240	1·622±·035	4·85±·09	2·51
·318	1·747±·041	4·63±·14	2·22
·373	1·853±·034	4·47±·08	2·07
	Solutions changed. Grown for 77 days		
·145	1·766±·073	6·79±·31	3·24
·230	2·298±·086	7·33±·25	2·95
·309	2·763±·070	8·33±·25	2·82
·364	2·949±·104	7·81±·19	2·69
	Solutions changed. Grown for 99 days		
·149	2·123±·13	8·51±·31	2·64
·235	2·843±·09	9·02±·25	2·51
·316	3·310±·07	10·22±·26	2·37
·373	3·358±·12	9·15±·28	2·20

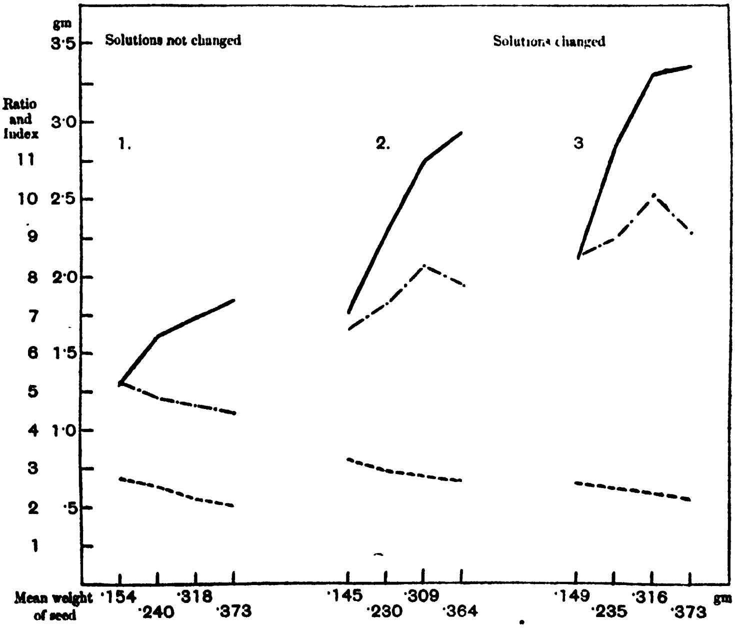

Fig. 5. Harbinger Peas grown from seeds of known individual weights grouped into quartiles. Mean figures for 13 or 14 plants in each case. 1, 2. Aug. 31st–Nov. 16th, 1920. 3. Aug. 31st–Dec. 8th, 1920.

———— Total dry weight. —•—•—•— Shoot/root ratio. - - - - - Efficiency index.

root to make the fullest use of the extra supplies of nutriment presented with the heavier seeds. In other words the improvement in the growth capacity of the young shoot and root with increasing size of seed is not fully commensurate with the additional food supplies laid down in the cotyledons or endosperm, and this is reflected in a slightly lower rate of working as revealed by the decreased efficiency indices.

A comparison of plants from the same sowing, grown under similar conditions with plenty of food, but harvested at different times shows that the efficiency index per cent. per day of plants from the same size of seed decreases as time goes on (see Fig. 5, curves 2 and 3). This is in full accord with the earlier experiments (2) which showed that the efficiency index over weekly periods first rises to a maximum and then falls, so that the average rate of increase over the whole period is less than that attained in the earlier part of the life of the plant. The relative development of shoot and root are to some extent influenced by the initial weight of the seed, the effect partly depending on the abundance or scarcity of food. With barley grown with a limited food supply for 67 days the ratio between shoot and root was about 2, whatever the weight of the seed, both shoot and root developing at equal rates except with specially heavy grains weighing over ·60 gm., when the ratio rose to 2·6 (Fig. 2). With plenty of food for 162 days the ratios for medium sized seeds approximated to 5–5·4 (omitting the single plant from one seed below ·02) rising to over 6 with very heavy seeds. The rise in the ratio with the improved food supply is significant, indicating that when deficiency of food is not a limiting factor the shoot of barley tends to increase more rapidly than the root; this is chiefly evident when the external source of supply is abundant, but is shown to some extent when the reserves stored in the endosperm are large, as occurs with very heavy grain. Peas, on the contrary, gave quite different results. With a limited supply of food the shoot/root ratio steadily decreased as the seeds became heavier, under such conditions the roots being able to make better progress than the shoots. When, however, plenty of food was available the ratios showed a corresponding rise, greater aerial growth being made with a more limited root system, probably indicating a greater assimilatory activity in the plants from the heavy seeds. This again was followed by a decrease with the heaviest seeds, no corresponding decrease being shown with barley (Fig. 5). The pea plants in the latter case were not very much heavier than those from the next grade of seed, especially after prolonged growth, and the fall in the shoot/root ratio suggests that the advantageous effect of the heavier seed persists in the root after it has ceased to tell with such effect on the shoot. This is borne out by the fact that the fall is more strongly marked after a longer period of growth (cf. plants harvested on Nov. 16th and Dec. 8th, Fig. 5). As time goes on the shoot/root ratio increases, but the rise in the plants from the heaviest seeds is considerably less than in those from rather lighter seeds.

CONCLUSIONS.

The results thus obtained with peas and barley grown under controlled conditions from seeds of different weights, go to support the view of the advantage generally to be gained with crops that are short lived, as spring cereals, roots, peas and beans, by sowing heavy well filled seeds instead of corresponding numbers of light seeds even though equally well filled. The data obtained from these experiments do not show the comparative results of sowing equal volumes of the two classes of seed, *i.e.* equal number of bushels per acre.

The results further indicate that with a longer growing period the advantage tends to diminish owing to the less rapid rate at which the percentage increase of dry weight takes place with the heavier seeds, thus providing an explanation of the field results with perennial crops such as lucerne, in which the weight of the individual seeds sown is apparently immaterial. There is every justification, therefore, for laying emphasis on the importance of the quality and weight of seed, as larger crops may be anticipated from the use of larger and heavier seeds, more especially if the season is unfavourable, though even initially poor plants may make very satisfactory progress if the season is exceptionally favourable to the growth of any crop.

SUMMARY.

(1) Field experiments, especially in America, have indicated that, with annual plants at least, better crops are usually obtained by the use of heavy seed in preference to light, whether equal numbers of bushels or equal numbers of seeds are sown per acre.

(2) Water culture experiments with peas and barley have shown that there is a steady and considerable rise in the dry weight of the plants as the initial weight of the seed increases. This occurs both with a limited and abundant food supply.

(3) The efficiency index (rate per cent. increase per day) falls gradually as the weight of the seed rises. With prolonged periods of growth this tends ultimately to counterbalance the initial advantage gained by plants from the heavier seeds, but with annual crops as cereals, peas, etc

harvesting occurs before this equilibrium is reached, leaving the advantage with the heavier seeds.

(4) The relative development of shoot and root is to some extent influenced by the initial weight of the seed, but may vary with the species and with the amount of available food.

(5) The results lend support to the growing agricultural practice of advocating the use of large heavy seed, especially with annual crops. The advantage in the case of perennials would appear to be less, if any, but this has not been determined by laboratory experiments.

REFERENCES.

(1) BLACKMAN, V. H. (1919). *Ann. Bot.* XXXIII, 353–360.
(2) BRENCHLEY, W. E. (1920). *Ann. App. Biol.* VI, No. 4, 211–244.
(3) BOLLEY, H. L. (1901). *Eleventh Annual Report*, North Dakota Agric. Exp. Stat.
(4) BOSS, A. (1893). Minnesota Agric. Exp. Stat. *Bull.* 31, p. 213.
(5) DESPREZ, F. (1922). *Journ. Agric. Pratique*, T. 1, No. 7, pp. 141–143.
(6) FRUWIRTH, C. (1917). *Fehling's Landw. Ztg.* LXVI.
(7) GEORGESON, C. C., BURTIS, F. C. and SHELTON, W. (1891). Kansas Exp. Stat. *Bull.* 30, p. 187.
(8) GEORGESON, C. C., COTTRELL, N. M. and SHELTON, W. (1891). *Ibid. Bull.* 20, pp. 7–9.
(9) GEORGESON, C. C., BURTIS, F. C. and OTIS, D. H. (1893). *Ibid. Bull.* 40, pp. 55–57.
(10) —— (1896). *Ibid. Bull.* 59, pp. 98–99.
(11) HAYS, W., HOVERSTAD, T. A., PENDERGAST, W. W. and BOSS, A. (1896). Minnesota Agric. Stat. *Bull.* 50, pp. 322–325.
(12) KIDD, F. and WEST, C. (1918–19). *Ann. App. Biol.* V, pp. 1–10 [illegible]2–142, 157–170, 220–251; VI, 1–26.
(13) LEHMANN, J. (1869). *Zeitschr. d. landw. Vereins in Bayern.*
(14) REED, H. S. (1919). *Amer. Journ. Bot.* VI, 252–271.
(15) SOULE, Q. M. and VANATTER, P. O. (1901). Tennessee Agric. Exp. Stat. XIV, *Bull.* 3, pp. 3–6.
(16) VOELCKER, J. A. (1901–2). *Reports on Field and Pot Culture Experiments*, Woburn Experimental Station, pp. 48–51; 1902, pp. 34–35; 1903, pp. 29–30.
(17) —— (1905–6). *Ibid.* pp. 22–23.
(18) WHEELER, W. A. and BALZ, S. (1906). South Dakota Agric. Coll. *Bull.* 96, pp. 59–60.
(19) ZAVITZ, C. A. (1905). *Ann. Rep.* Ontario Agric. Coll. and Exp. Farm, pp. 170–172.

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NOTES ON THE CONTROL OF "CUTWORMS" BY POISONED BAIT

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(With 3 Text-figures.)

ABROAD, and notably in the U.S.A., the destruction of Agrotid caterpillars (Cutworms) by means of a poisoned bait is generally recognised as the standard treatment, but in Britain, although entomologists appear to have used the method from time to time, the published records of their results are too scanty to justify any final conclusions. The abnormal abundance of Agrotid larvae in August and September of 1921 gave an opportunity for carrying out a further series of experiments which are here described, chiefly for the information of entomologists, since it is recognised that there are still difficulties to surmount before the method can be advised to the farming world in general.

The use abroad of poisoned bait against Cutworms was the direct outcome of its success in dealing with plagues of locusts or grasshoppers and it would seem that in the majority of cases the same mixture has been used. Little information is, however, available as to the precise part played by some of the constituents and it seemed wise, therefore, before proceeding to field trials to discover in the laboratory whether the Cutworms (*Euxoa* (*Agrotis*) *segetum*) had any preference for any particular chemicals or flavours. The American bait consists of moist bran as a base, flavoured with cane molasses and lemon, and rendered poisonous with Paris Green. Since moist bran in practice is a very convenient base it was retained in these experiments and when flavoured with one of the substances under trial, was tested according to one or other of the following methods.

SERIES I.

The floor of a cage 2 ft. square was divided by wooden partitions 1 in. high into four shallow compartments of equal size. The compartments were filled with peat moss litter so as just to bury the partitions and three different substances under test were placed one in each compartment in the corner furthest from the centre (Fig. 1). In the corresponding corner in the fourth compartment was placed a potato or mangold in accordance as to whether the larvae under test had been obtained from a potato or a mangold field. From 50 to 100 larvae were then allowed to bury themselves in a shallow dish completely filled with peat litter, which was placed exactly in the centre of the cage floor resting on the intersection of the four partitions and a little further peat was sprinkled round the outside of the dish so as to make it possible for the larvae to crawl out easily. As Agrotids are chiefly nocturnal feeders the cage was left for the night, care being taken to prevent external influences such as light or wind from affecting any one side more than the other. The following morning the number of larvae in each compartment was counted, the assumption being that after feeding the larvae would burrow into the peat upon which the food rested and would be prevented by the partitions from straying into one of the other compartments in which they had not fed. It was also assumed that, as the natural food was always present, the larvae would not feed on the baits unless they were at least as attractive.

The results from three of these experiments were as follows:

(The figures represent the numbers of larvae, reduced to percentages, found in the compartment in which the particular substance named had been placed.)

Exp. 1.	Natural food (mangold)	43 per cent.
	Citric acid and moist bran	22 ,,
	Amyl acetate and moist bran	22 ,,
	Molasses and moist bran	13 ,,
2.	Natural food (mangold)	18 ,,
	Molasses and moist bran	29 ,,
	Chopped turnip leaves	31 ,,
	Amyl acetate and moist bran	22 ,,
4.	Natural food (mangold)	19 ,,
	Molasses and moist bran	26 ,,
	Chopped turnip leaves	30 ,,
	Amyl acetate and moist bran	25 ,,

A number of similar experiments were made, but they need not be reported as no deductions could be made from them except that the larvae appeared to show no very marked preference. It seemed to be more or less a matter of chance whether they fed upon the bran bait or upon their natural food plant.

A fresh type of cage was then evolved to deter still further the larvae from wandering, since it was considered that the results obtained in the first series of experiments might have been vitiated by the movement of larvae after they had fed.

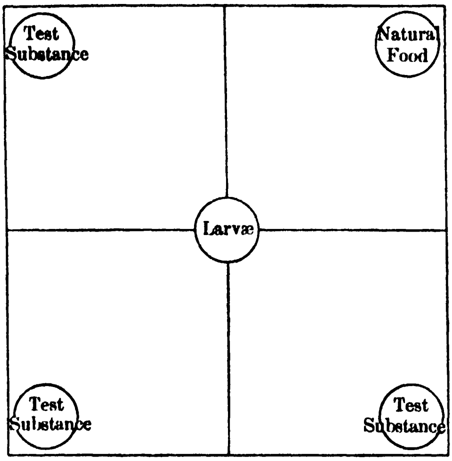

Fig. 1.

SERIES II.

A cheese tub was fitted with a central partition dividing the tub into two compartments with the exception of a space of about 2 ins. at each end of the partition (Fig. 2). The whole floor was covered with peat and the natural food placed on one side of the partition near the centre, the moist flavoured bran in a similar position on the other. Equal numbers of larvae were placed in each of the two compartments and the experiment was left until the following morning, when the numbers of larvae

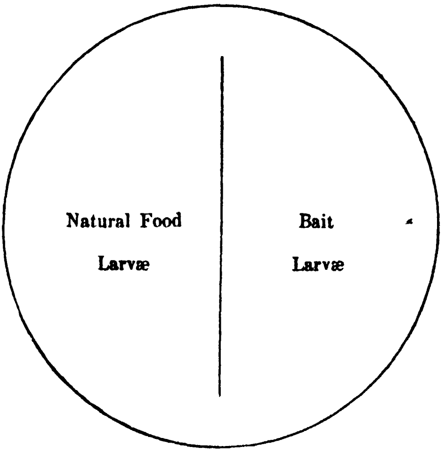

Fig. 2.

in the compartments were counted. In this instance it was assumed that if a larva was content with the food in its own compartment it would make a meal and when satisfied remain near by; that otherwise it would wander to the opposite compartment in search of food more palatable. It was believed, for instance, that unless a larva was satisfied with "flavoured" bran, it would go in search of mangold or turnip before making its nightly meal.

The following results are typical of those obtained by this series:

Exp. 6.	Turnip	73 per cent.
	Clove oil and moist bran	27 ,,
9.	Turnip	50 ,,
	Beet molasses and moist bran	50 ,,
12.	Mangold	52 ,,
	Molasses, colza and moist bran	48 ,,
14.	Mangold	60 ,,
	Colza and moist bran	40 ,,

In the case of clove oil a definite aversion could be deduced but otherwise the larvae again appeared to exhibit no strong preference.

At this point it may be well to state that some of the larvae which had been discovered on the "bran" side in the various experiments were dissected and all showed evidence of having actually fed upon the bran.

SERIES III.

In view of the ambiguous results of these two series of experiments a third form of cage was designed in order to impose on the larvae the necessity of choosing definitely between their own food plant and the bran bait. The apparatus in this case again consisted of a cheese tub but with a high partition passing from the circumference of the tub diametrically to within 6 ins. of the side (Fig. 3). Opposite the free end of the partition a small compartment about 4 ins. square was constructed with a narrow opening directly facing the partition end. The tub was thus divided into three compartments, two large and one small, intercommunicating by a narrow space at the free end of the main partition. A thickness of 3 or 4 ins. of peat litter was then placed on the floor of the tub and on one side of the main partition a mangold, potato, etc. according to the field from which the larvae under test were obtained. On the other side a handful of the flavoured bran mixture was partly buried in the peat. A trail of the same bran mixture was then laid from the main mass to the opening of the small compartment or "box," where it mingled with a trail of chopped mangold leading to the mangold root itself. From 40 to 50 larvae were then placed in the box in peat, in which they buried themselves until evening. On moving to search for food, they could only leave by the opening where they were at once presented with the bait and their own natural food, in such a manner that they could not fail to realise the presence of both. It was assumed, as actually occurred, that they would follow one or other trail until they reached the natural root or the bran bait, where they would complete their meal and go to rest in the peat litter near by. Any tendency to wander after feeding would be likely to cause but a small error, since the chances of larvae finding the relatively small opening between the two main compartments would be few. The likelihood was, however, further reduced by piling peat to a considerable depth at the fixed end of the partition and reducing it so as to be barely sufficient to cover the larvae in the other half of each compartment. Since the tendency of the larvae is to bury themselves, this arrangement of the peat would tend to discourage them from leaving the part of the box in which the food was placed.

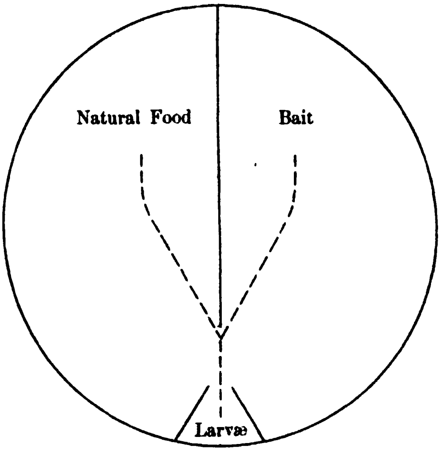

Fig. 3.

The following results were obtained from this form of experiment, the numbers again being reduced to percentages. It may also be mentioned that in most cases a few larvae remained in the box, probably because they had recently fed or because they were changing their skins and therefore unwilling to eat. These larvae are not included in drawing the percentages.

Exp. 7.	Turnip	52	per cent.
	Beet molasses and moist bran	48	,,
9.	Turnip	66	,,
	Clove oil and moist bran	34	,,
11.	Mangold	55	,,
	Colza, molasses and moist bran	45	,,
14.	Mangold	41	,,
	Colza and moist bran	59	,,
15.	Mangold	53	,,
	Moist bran (unflavoured)	47	,,
17.	Mangold	47	,,
	Moist bran and salt	53	,,
19.	Moist bran	82	,,
	Peat but no food	18	,,
20.	Mangold	85	,,
	Peat but no food	15	,,
21.	Mangold	61	,,
	Pimento and moist bran	39	,,
22.	Mangold	53	,,
	Mace and moist bran	47	,,
23.	Mangold	47	,,
	Coconut oil and moist bran	53	,,
26.	Mangold	52	,,
	U.S. bait: lemon, molasses, bran (moist)	48	,,
27.	Mangold	50	,,
	U.S. bait: lemon, molasses, bran (moist)	50	,,
28.	Mangold	64	,,
	Linseed oil and moist bran	36	,,
29.	Mangold	57	,,
	Dandelion leaves	43	,,

In this series of experiments, it will be noted that clove oil (Exp. 9) again showed some signs of being distinctly repugnant and the same was perhaps the case with Pimento (Exp. 21). In the other experiments, except Nos. 19 and 20, preferences are not strongly marked and although in certain cases—Exp. 14—with colza oil, some attractiveness on the part of the oil may be argued, they are either contradicted by other experiments or are not beyond the limits of experimental error. In the case of Exps. 19 and 20 some evidence as to the dimensions of this error are obtained, the figure working out at about 20 per cent. and it is worth noting that this conclusion is supported by the next series of experiments.

Attention may also be drawn to Exps. 26 and 27, where the bait, apart from the absence of poison, was identical with that used in America against Cutworms. It will be noticed that no evidence is given of any special attraction from the use of lemon and molasses, while summarising the whole series it appears that the addition of flavours does not add to the attractiveness of moist bran, which clearly is not far different from that of the normal food plant.

SERIES IV.

While the foregoing experiments were in progress, attempts were being made to discover a dye which could be used with the bran, and on being eaten would distinguish the larva in question without causing it material damage and without affecting the attractiveness of the bran bait. It was undesirable that the larvae should be dissected in order to detect the dye, which therefore to be effective had to give a very pronounced coloration to the alimentary canal. Finally, a sample purchased as B.P. liqu. cochineal was found satisfactory and was used in a considerable number of experiments, but it may be noted that after the original sample had been exhausted it proved impossible to obtain a similar dye again. Several dyes made according to the B.P. formula were both purchased and also made up in the laboratory, while innumerable variations were also tested, but none were found to approach the standard set by the first sample, which was indeed almost ideal. A number of aniline dyes were also used with varying results, reds being always the more satisfactory, but none was so generally useful as cochineal, with which this series of experiments was in the main carried out.

In regard to method, a number of experiments with cochineal-dyed bran were conducted with the type of cage described in Series III (Fig. 3) in order to check the error of this apparatus. By counting all the red stained larvae which were found on the side of the partition containing the natural food and by assuming that any unstained larvae found on the bran side had fed upon mangold, etc. (and then crossed over) it was possible to calculate the maximum error, which worked out at approximately 20 per cent. This figure is certainly too high, owing to the inclusion of all unstained larvae found on the side of the cage containing red bran, since a number of such larvae may well have been those which owing to being about to change their skins, were unwilling to eat. Accepting, however, an error of 20 per cent. it will be seen that the conclusions derived from the tests are not substantially affected.

The method was then further developed, so as to approach more nearly the field conditions, by the removal of the main partition in the third type of cage, leaving only the small box-like compartment. Peat to the thickness of 3 or 4 ins. was then laid on the bottom of the cage and potatoes or mangolds buried in it at regular intervals, leaving a small portion of their surfaces exposed above the peat. Bran dyed red with cochineal, flavoured or not as the case might be, was then sprinkled thinly over the surface of the peat. The larvae, 50 in number, were placed as before in the compartment until the following day, when they were recovered from under the peat and examined to discover which had eaten the bran.

These experiments merely served to confirm the conclusions already formed from previous experiments, to the affect that the larvae were as willing to eat bran as their proper food and that although the addition of various flavouring materials to the former might reduce its attractiveness, in no case did they add appreciably to it. In the experiments with dyed bran about half the larvae showed that they had fed upon bran, while the other half showed no evidence of having done so. It was therefore considered that if a poisoned bran bait could be distributed in a field so that the larvae were as likely to discover it as to find their natural food, then about half the caterpillar population in the field should be killed.

Since at this time the season was advancing, the search for more attractive baits was abandoned, and field trials with moist bran unflavoured but rendered poisonous were arranged. Before giving the results of these trials, a list of the flavouring materials tested may be inserted in order that those who conduct similar experiments may know which were found ineffective. It may also be explained that, apart from the American experiments, there was nothing to suggest what substances were likely to prove effective and the flavouring materials were selected, more or less at random, in the hope of finding something sufficiently marked in attractiveness to justify a detailed investigation and analysis. The time available was, unfortunately, insufficient to enable any organised trials with pure chemicals to be made, and importance was attached to the necessity of a potentially attractive substance being readily available to the cultivator through the ordinary channels of purchase.

Beet molasses
Cane molasses*
Clove oil
Colza oil
Linseed oil
Coconut oil
Aniseed oil
Cinnamon
Nutmeg
Mace
Pimento
All spice
Amyl acetate
Allyl isothiocyanate
Apple chopped
Lemon juice*
Turnip leaves chopped
Dandelion leaves
Sodium chloride*

Those found effective against Cutworms or Grasshoppers in America are marked with an asterisk.

SERIES V. FIELD TRIALS.

The first trial was carried out on August 22nd at Rothamsted in a mangold field evidently, but not severely, attacked, almost every root showing signs of damage, but comparatively few having been killed. An area of approximately 4 rods was marked out and was dressed in the afternoon with the following mixture:

Bran dyed with cochineal	½ lb.
Paris Green	½ oz.

the mixture before use being moistened with water until the particles of bran just failed to adhere to each other. The bait at this stage feels damp to the hand but can still be easily sown broadcast. On a part of the experimental area the bran was sown down the rows and on another it was scattered uniformly, but this difference may be neglected, as subsequently it was found to have no effect on the ultimate result. On the following afternoon a portion of the treated area was examined with great care and every larva found was gathered, in all 68. Of these, four were dead, while 24 showed signs of arsenical poisoning. The four dead larvae proved on dissection to have eaten freely of the poisoned bran. The remaining larvae were kept to discover the numbers which died or recovered. On the day following a similar portion on the plot was searched and produced 60 larvae, of which 15 were dead and 12 evidently poisoned. As before, these were kept until a final result was obtained on August 25th as follows:

1st lot—living, 41; dead, 27.
2nd lot—living, 29; dead, 31.

Summarising, out of a total of 128, 58 were destroyed by the treatment, approximately 45 per cent.

This percentage is probably a little too low, as it is exceedingly easy to overlook dead larvae, which shrivel rapidly on a hot day and are very inconspicuous in the soil or at times even upon the surface, for they were found in either situation.

The measure of success obtained in this small trial and also the fact that the results corresponded so nearly with the probable results as calculated from the laboratory experiment, made it desirable to extend the work to trials under normal farm conditions. This took place at Symonds Hyde, Hertfordshire, first on August 29th and then on September 6th on a field partly mangolds and partly swedes, which was being seriously damaged by Agrotids (practically all *A. segetum*). One and a half acres of mangolds and one acre swedes were dressed with bran 56 lbs. to Paris Green 3 lbs. moistened with water as in the previous experiment, the amount of water needed being approximately seven gallons. The work was done by farm labourers who sowed the plot broadcast, only covering two and a half acres instead of three as was originally intended. The bait was sown between 4.30 p.m. and 5.30 p.m. summer time, but the day was hot with a drying wind and much of the bait must have become very dry before the night. On August 31st the field was examined, small areas about two square yards in different parts being searched and every larva found removed. No difference in result between the swede and mangold areas was detected and on September 2nd, until which day all the living larvae had been kept, the final result was recorded as follows:

Larvae living	170
Larvae dead	144

The percentage thus again works out at about 45 per cent. killed.

On September 6th a further trial was made, partly to test a mechanical method of distributing the bait and partly to discover if lead arsenate could be substituted for Paris Green. A Paris Green mixture was made up as before, while in the case of lead arsenate 6 lbs. of paste (20 per cent. As_2O_5) were used to each 56 lbs. of bran. Both lead arsenate and Paris Green were applied with a manure distributor of the Disc type (manufacturer, J. R. Wallace) which did the work well and with hardly any clogging. Some four acres in all were dressed as follows: one acre swedes which had previously been dressed on August 29th and one acre swedes not previously treated, both with Paris Green; two acres swedes (not previously treated) with lead arsenate. The field was again visited on the 9th, and as time was limited a collection of larvae was made on two sample areas of four square yards in each plot with the following results:

(*a*) *Swedes twice dressed.*

Larvae living	40
Larvae dead	59

i.e. approximately 59 per cent. of the larvae were found to have been killed.

Much reliance must not, however, be placed upon this figure, for while some larvae had been killed by the second dressing, others had evidently been dead for some days and had succumbed to the first dressing. All of the latter would not, however, be recoverable after so many days, and therefore the figure is too high to represent the results of the single dressing and too low for the two dressings.

(*b*) *One acre swedes once dressed Paris Green.*

Larvae living	75
Larvae dead	40

In this case approximately 35 per cent. of the larvae only were killed, a result which may in part be due to the fact that the collection was made very hurriedly.

(*c*) *Two acres swedes once dressed with lead arsenate.*

Larvae living	91
Larvae dead	8
Larvae poisoned but not killed ...	10

As lead arsenate, at this strength at all events, was obviously ineffective, the ten poisoned larvae were not retained to obtain final figures as to mortality. As a check on the above figures and to show that the larvae were not dying from any natural disease, one of the counts from a control plot, in this case swedes, may be mentioned, the area named being four square yards:

Living, 59. Dead, 0.

At this point the swede and mangold experiments were left, as the damage to the roots had already been sufficiently great to invalidate any results to be obtained by weighing the crop.

On the same farm a field of potatoes had been ruined by the Agrotids and although owing to their great depth in the soil it seemed almost hopeless to deal with them by the bait method, a small preliminary trial was carried out. It proved that a few of the larvae at all events came to the surface and died after eating the bran but circumstances did not permit of the trials being concluded and no figures can be given.

CONCLUSION.

The foregoing experiments show that the bran bait method of dealing with Cutworms is very fairly effective in the case of mangold and swede fields, one dressing destroying about 45 per cent. (one plot fell to 35 per cent.). The search in the laboratory for substances specially attractive to Agrotid larvae failed to produce anything better than moist bran, which appeared to be taken practically as readily as the natural food, so that on an average 50 per cent. of the larvae tested fed upon bran and 50 per cent. upon their own proper food. The approximation of this figure to the number destroyed in the field experiments is curious and to some extent the two types of experiment support each other. This is explained, not by presupposing a preference for bran by 50 per cent. of the larvae, but by assuming that the larvae have no preference and have an even chance of meeting with bran or their own food.

As regards the future of the poisoned bait method in practical agriculture, the chief difficulty lies in the possibility that the poisoned bran might prove a danger to poultry, game and other birds, or even it has been suggested, to man through eating poultry and game which themselves had been feeding on the bran or poisoned larvae. All those who have experimented in the U.S.A. or Canada have satisfied themselves that this danger is not a real one and the method is now recognised as standard, the only precautions advised being those relating to the mixing tubs or to the labour engaged in distributing the bait. In the experiments previously recorded, the dressing appeared very slight, so that, without a most careful examination, it would be impossible to discover from the bran on the surface which areas had been dressed and which had not. It seemed almost impossible that so small a dressing could prove a danger. On the other hand the amount of Paris Green used per acre was 1 lb. while in America ⅓ lb. per acre is considered sufficient. There appears to be no evidence to show the quantity of Paris Green which is toxic to vertebrates, but on the assumption that each one grain of arsenious oxide in combination would form a poisonous dose, it would seem that in order to be poisoned a bird would have to eat every particle of bran on 1½ to 2 square yards (or from 4½ to 6 square yards dressed on the American formula). This possibility, though unlikely in the circumstances in which the bait is used, cannot be altogether ruled out and it does not seem wise to recommend the treatment for application in agriculture except in special circumstances. It should also be noted that no opinion is offered as to the conditions under which the use of the bran bait would be an infringement of the Protection of Animals' Act, 1911, Section 8.

On all accounts, therefore, it is evidently desirable that some poison should be discovered which is sufficiently toxic to the larvae but in the quantities used would prove harmless to birds and other vertebrates. The discovery of some such insecticide should not prove impossible.

In conclusion, the authors desire to thank Sir John Russell of the Lawes Agricultural Trust for permission to experiment on a field on the Rothamsted farm, and also to Mr T. G. Walker of Symonds Hyde Farm, for the assistance he rendered both in placing his fields at our disposal and for his generous co-operation throughout the work. Dr Imms of Rothamsted also kindly assisted us in regard to suitable substances for use with the bran.

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FUNGI ASSOCIATED WITH "DIE BACK" IN STONE FRUIT TREES. I.[1]

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(With Plates XI–XIV.)

INTRODUCTION.

THE investigation as to the various causes of "die back" to which all stone fruit trees are so prone, was started with the study of a serious wilt and "die back" of Prince of Wales plum. This disease was first brought to the notice of the writer in 1919, by Mr Lobjoit, Controller of Horticulture. He described the disease as causing very serious damage, in Middlesex, to plum trees of the Prince of Wales variety, so much so, that there was considerable risk of this good market variety going out of cultivation. The investigation was then extended to all forms of "die back" in stone fruits, to see if "die back," in general, could be attributed to biological strains of one and the same fungus. Results, however, have shown that this is probably not the case. Four distinct fungi, easily distinguishable from one another, have been isolated from diseased tissues in "die backs" occurring on various kinds of stone fruits such as peach, apricot, nectarine, pear, different varieties of plums and also plum and apple stocks.

Three fungi only will be dealt with in this paper, namely *Cytospora* and *Eutypella*, on which a considerable amount of work has been done already by other investigators, and a new species of *Diaporthe*, which, so far, has not been recorded as being the cause of, or being associated with, "die back" in stone fruit trees in this country.

The causes of the so-called "die back" of fruit trees are still little understood, in spite of a considerable amount of literature which has accumulated on the subject. There appears to be a good deal of confusion owing to different investigators ascribing "die back" on different hosts to different organisms, and also to the fact that most, if not all of the

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fungi described, are alleged to have two distinct stages in their life-history, namely a pycnidial stage, in the form of *Phomopsis*, *Fusicoccum*, *Phoma* or *Cytospora*, and a perithecial or valsoid stage.

Massee in 1902(35) investigated "die back" in young nursery fruit trees, more especially plum, apple, and pear, and attributed the disease to the fungus *Eutypella prunastri*. He also claimed to have brought about infection by applying spores of the conidial stage to lenticels in damp weather. There is, however, considerable doubt as to whether *E. prunastri* occurs on cultivated plum in this country, and if it does, it is far from common. For the last two years, during which a considerable number of specimens of "die backs" on various hosts have been examined, no *Eutypella* has been observed on cultivated plum, peach, apricot or apple, in spite of careful search.

True *E. prunastri*, however, does occur on wild sloe, wild plum, wild cherry, crataegus, etc.(46). *Eutypella*, as described by Massee, has one-celled ascospores, whereas all the perithecia found during the present investigation, with one single exception, contained bicellular ascospores. Since Massee's publication, it seems to have been generally accepted that "die back" is caused either by *E. prunastri* or by *Cytospora leucostoma* or *C. rubescens*. Both Cooke(5) and Saccardo give *C. rubescens* as the conidial stage of *Eutypella*.

Epidemics of "die back" attributed to *E. prunastri* have been reported from time to time. In 1902, in the *Woburn Report*(60), an interesting description is given of a serious outbreak of "die back" in a plantation of young plum trees on the estate, in which 41 per cent. of the trees, from four to nine years old, were killed in the course of three to four years. Different investigators attributed the disease to different causes, including *Eutypella*. Some of the symptoms described agree very closely with those observed by the present writer in cases of "die back" due to *Diaporthe*. This outbreak seems to have been successfully stopped by cutting away all diseased parts, and by removing all dead or dying trees. In 1910(51) another serious outbreak of *E. prunastri* was reported as occurring in Kent, Worcestershire and Herefordshire, but no description of the fungus was given.

Besides *Cytospora* and *Eutypella*, other members of the genera *Phomopsis*, *Fusicoccum*, *Phoma*, etc. with or without a known ascigerous stage, have been recorded as causing disease on widely different host plants, some—more especially in the case of hard-wooded plants—associated with "die back," others as causing rots, etc. Further extensive investigation, together with cross inoculations, alone will show whether the organisms occurring on closely allied hosts, the different stages of which have been described by different investigators under various names, may not be strains of the same fungus. It is not possible to give a complete list, but references to the more important and better known diseases caused by fungi belonging to, or closely allied to, the above genera, are given in the bibliography.

(1) CYTOSPORA.

After years of controversy (1, 14, 16, 17, 34, 55, 56), between pathologists in Germany, there appears to be no doubt that Aderhold (1) was able to prove by inoculation experiments that a *Cytospora*, with a valsoid stage, having gained access as a saprophyte on dead twigs, etc. can spread through the living tissues as a parasite and cause "die back" of cherry in Germany. Rolfs (48) in America also made successful cross inoculations with both pycnospores and ascospores of *Cytospora* on peach, plum, apricot, red cherry, and wild cherry.

On the other hand, Wormald (62), working on cherry "die back" in this country, failed to bring about the disease by inoculation, although he always found *C. leucostoma* associated with "die back" on cherry. He also failed to find mature perithecia.

It is difficult to say whether this strain of *C. leucostoma* is the same as the *Cytospora* with the valsoid stage occurring in Germany and America, more especially as perithecia of *C. leucostoma* have not been observed so far on stone fruits in this country.

Belgrave (4) has described *Cytospora* as occurring on Victoria, Prince of Wales and Pond's Seedling plums, but he did not make inoculations with pure cultures, and was not able to find mature ascospores. He found that "die back" was followed by the formation of pycnidia with red gelatinous tendrils of the genus *Cytospora*, "the members of which are recognised as conidial stages of various species of *Valsa*."

In the opinion of the writer, it is doubtful, judging from first-hand experience, and the failure of the above workers to bring about infection by inoculation, whether, with the exception of cherry and peach, the strain of *Cytospora* found in this country associated with "die back" in stone fruits, is really the primary cause of the disease. During the present investigation, the perithecial stage of this fungus has never been found, and in no instance have cultures of ascospores from valsoid perithecia on cultivated fruit trees, given rise to the conidial stage of *Cytospora*. Valsoid perithecia do occur in considerable numbers on branches suffering from "die back" on different hosts; but these perithecia, which have always shown two-celled ascospores, belong to *Diaporthe*, a fungus entirely distinct from *Cytospora* or *Eutypella*, both of which have unicellular acospores.

A detailed description of *C. leucostoma* is to be found in Wormald's paper (62) on "The *Cytospora* disease of the Cherry," and this description agrees in every detail, as far as the morphology of the pycnidial stage of the fungus is concerned, with the *Cytospora* observed on peach and different varieties of plum during the present investigation.

In the case of plum trees, *Cytospora* is very frequently present on the dead parts of trees suffering from "die back," but it appears to be secondary, and the cause of the mischief can generally be traced to a region lower down, where the tissues are still alive, but more or less moribund. The bark in this region may or may not show definite signs of disease, but if portions are kept under moist conditions in a petri dish, the *Phomopsis* stage of a *Diaporthe* will develop in the course of a few days. A very striking instance of this occurred at Cookham Dean, in a young plantation of standard trees of the Prince of Wales variety of plum, a considerable number of which had been killed. *Cytospora* was found on portions of the dead branches sent in for examination. The nursery was then visited and the dead and dying trees carefully examined. The trees had been grafted at soil level, and the scion taken up to form the stem of the young standard trees, and examination at the base of the stem in the region of the graft showed typical *Diaporthe* lenticel-like pustules on nearly all the trees. Chips of bark from near the graft from a number of the trees on being kept a few days under moist conditions, developed the typical *Phomopsis* stage, and in a few instances the *valsoid* stage of *Diaporthe*.

The trees had evidently died from the graft upwards, as the stocks were alive and apparently healthy.

So far, inoculations on Prince of Wales plum with pure cultures of *Cytospora* isolated from the same variety of host, made by inserting spores under the bark of one year old shoots, have given negative results. The wounds remained healthy, showed no signs of infection and healed up quite as well as the controls. Two sets of inoculations were made, one at the end of March, 1920, and the other at the end of May in the same year. The trees have now been under observation for rather more than two years.

However, so little is known, as yet, as to the conditions conducive to infection by *Cytospora* and other members of this difficult group of Valsaceae, that these negative results must not be taken as conclusive.

There are grounds for believing that *Cytospora* may be weakly parasitic and infect tissues which are not quite normal owing to other causes, such as bad cultural conditions, insufficient moisture, excessive or indifferent pruning, bad drainage, etc. or it may gain access into the host as a saprophyte living for a time on dead twigs, spurs, etc. and from thence spread to the living tissues. This was proved to be the case by Aderhold with cherry "die back" (1) and Hubert with *C. chrysosperma* on certain forest, shade, and ornamental trees (30).

There may be and probably are many strains of *Cytospora*, some more virulent than others, some capable of forming perithecia, others not, and so on. So far, no attempt has been made to differentiate between strains of *Cytospora*. One point, however, has been observed by Belgrave, Aderhold, and the present writer, and that is, the variability of colour in the tendrils or globular exudations of *Cytospora*, from deep red to pale yellowish pink. This variation does not seem to depend, as do the size, shape and length of the tendrils, on the amount of external moisture present.

Peaches, grown out of doors, when attacked by *Cytospora*, show definite cankers, accompanied by exudation of gum on the older stem and branches (Pl. XI, fig. 2). On the younger growth the fungus girdles the shoot, resulting in rapid wilt and "die back" of the parts affected.

(2) *DIAPORTHE PERNICIOSA.*

This fungus has proved to be far more prevalent on portions of various stone fruits suffering from "die back" than any other fungus so far met with. It has been isolated from "die back" on peach, peach stock, apricot, pear, apple stock type II (Malling), wild sloe, and the following varieties of plum, Prince of Wales, Pershore, Victoria, Coe's Golden Drop, Goliath, Greengage, various seedlings from the cross New Orleans × Victoria, seedlings of Victoria selfed, and other unnamed varieties sent in from various sources. Specimens of the disease have been received from Wisbech, Cambridgeshire, Surrey, Middlesex, Devonshire, Berkshire, Kent, Anglesea; and "die backs" have been reported from other fruit-growing areas.

The disease attacks trees of all ages, but the damage is more noticeable on young trees, when the disease generally kills the whole tree very rapidly after the first symptoms have appeared. After the examination of a considerable amount of material, the conclusions arrived at are: that the development of the fungus in the host tissues is slow; that infection occurs a considerable time—in some cases probably years—before any definite external symptoms can be seen; but that, once wilting has occurred, the affected areas die very rapidly, and the fungus completes its life-history as a saprophyte on the dead wood.

The first symptoms may be either rapid wilting and browning of the leaves during the growing season; or premature yellowing and fall of the leaves in autumn.

The bark at first looks healthy, and frequently no external symptoms can be seen before wilting sets in. In other cases the bark may take on a reddish tinge, or slightly sunken areas may extend longitudinally down the stem or branch. The smaller branches or stem instead of being cylindrical are inclined to be angular, or flattened, and this appearance becomes more accentuated as the disease progresses. This angular appearance is due to the uneven development of secondary thickening in the parts affected. The development of secondary thickening is arrested in the diseased areas, whereas normal growth continues in the parts of the periphery unaffected by the fungus. Pl. XI, fig. 5 shows a cross-section of a small branch, cut in December, in which the previous summer's secondary thickening has developed on one side only, and has been arrested round the rest of the stem. Infection in this case must have taken place during the previous winter or early spring.

Very frequently a considerable amount of callus is formed at the edges of the healthy tissue; this is most marked when the callus is formed on both sides of the sunken area, with the result that the bark is burst longitudinally down the stem, the wood laid bare, and the callus exposed, thus increasing the risk of secondary infection by other fungi and bacteria. Longitudinal splits of considerable length are of common occurrence on the branches of Prince of Wales plum and on the stems of young standard trees when grafted at soil level.

As the disease develops, transverse elongated excrescences form on the bark, which to the naked eye are very similar to, but much more numerous than, the undeveloped lenticels on healthy bark (Pl. XII, fig. 1). These excrescences are caused by the development of stromata of mycelial tissue immediately beneath or in between the external layers of cork. The bark is finally ruptured by the rapid growth of the stroma, and the spores are liberated from pycnidia embedded in the stroma, in the form of mucilaginous threads or tendrils, white or whitey buff in colour. These tendrils vary in length and thickness (Pl. XI, fig. 6). Under very moist conditions the spores may emerge as mucilaginous globules, which spread over the surface of the bark.

The spores are given off in damp weather. In dry weather the bark does not slit open so readily and the pycnidia may be unable to liberate the spores for an indefinite period, as long as external conditions remain the same. Under dry conditions the mucilage of the extruded tendril also dries up, and the tendrils become hard and brittle, but never appear to pulverise. The mucilage can be easily re-dissolved in water. Hence the spores of this fungus, like those of *Cytospora*, are probably not wind borne, but carried on the feet of birds, visiting insects, and by wood-boring beetles, or washed down the stem, or splashed from the soil and from tree to tree during heavy squalls of rain, cf. (28).

The perithecia mostly occur in the same stroma as the pycnidia, either singly or in groups, but in some cases the perithecia lie apart from the pycnidia and their necks emerge through bark which has not been previously ruptured by a pycnidial sorus.

The fungus kills the cortical tissues, phloem, cambium, and medullary rays, and penetrates the wood, causing considerable discolouration of the xylem. If a diseased stem is cut across before it is quite dead and dry, discoloured areas can be seen in the internal tissues, and the darkened medullary rays can be traced with the naked eye. When the tissues are very dry the discolouration is not so apparent. This discolouration is not limited to any particular area, xylem of all ages can be affected. The whole of the internal wood may be affected, or the discolouration may be restricted to wedge-shaped areas or scattered blackened spots or lines in the interior of the stem (Pl. XI, fig. 5). These areas extend longitudinally for a considerable distance. There is always more or less xylem discolouration in stems attacked by *Diaporthe*, but this discolouration must not be confused with that due to pruning scars which have not completely callussed over. Different varieties of plums vary within wide limits as to their capacity for healing over pruning scars.

In the case of *Diaporthe*, black wavy strands of cells filled with dark mycelium can be traced down the stem.

As observed by Goethe in the case of *Nectria*, and Wormald (62) in *Cytospora*, the mycelium of *Diaporthe* passes vertically through the vascular system (Pl. XIII, fig. 14) and horizontally through the medullary rays (Pl. XIII, fig. 15). In the medullary rays the mycelium passes from one cell to another mostly through the pits (Pl. XIII, fig. 15). The medullary ray cells are filled with coarse brownish mycelium, which becomes very much attenuated when passing through the pits; but although mycelium, both coarse and fine, frequently occurs in considerable quantity in the lumen of the vessels, the latter are rarely completely blocked by the fungus itself. Some of the older vessels are blocked with tyloses and yellow deposits, probably of the nature of wound gum, but the number of vessels affected is not large enough to account for the sudden wilt. The walls of the cells are discoloured, and show a certain amount of disorganisation; in the case of the vessels, the mycelium is able to penetrate the cell wall. The wilting and death of the shoot is due to the destruction of the phloem, cambium, and cortical tissues, and the disorganisation and blocking of the medullary rays, and not to the mechanical blocking of the vessels by the fungus itself.

The Fungus.

Diaporthe is an Ascomycete belonging to the Sphaeriaceae group of the Pyrenomycetes; family Valsaceae; with two distinct stages, the *Phomopsis* of pycnidial stage, and the *Valsa* of perithecial stage.

The bark of plum at an early stage consists of several layers of cork cells, and the mycelium sometimes penetrates between these layers, and forms knots of twisted hyphae, either between or immediately below these corky layers (Pl. XIII, fig. 10). These knots develop into the stromata in which the pycnidia develop. As growth proceeds a wavy line appears in the median region of the stroma. This line, the proliferous stratum of the pycnidium (Pl. XIII, fig. 10 *h*), consists of finer, more deeply staining hyphae than the surrounding coarse mycelium of the stroma. The upper and lower layers of the stroma split apart and the split forms the irregularly shaped chamber of the pycnidium, which is lined throughout with sporophores, bearing pycnospores. The number of chambers varies according to the size and shape of the stroma, but the dividing walls do not completely enclose each chamber, so that the pycnidium of *Diaporthe* can be distinguished from that of *Cytospora* which is much more definitely multilocellate.

Two distinct forms of pycnospores or conidia are found in one and the same pycnidium, as described by Diedicke and Grove (12, 18) in the genus *Phomopsis*, and by Harter and Field (25) in *Diaporthe batatis*, namely, long filiform hyalin spores (Pl. XIII, figs. 2, 7) definitely hooked at one end (rarely straight), thickening slightly in the middle region 25–28 $\mu \times 1\ \mu$, and called by Diedicke and Grove (12, 18) "b" spores, by Nietsche (43) stylospores, and by Marchal "basides" (38); and unicellular hyalin fusiform or elongate oval spores, 7–9 $\mu \times$ 2–3 μ, with two large and several smaller oil drops, the "a" spores (Pl. XIII, fig. 1).

The filiform "b" spores are given off from short, subulate (Pl. XIII, fig. 6 *a*), and the "a" spores from long, simple, cylindrical, or slightly subulate sporophores (Pl. XIII, fig. 6). The filiform spores are always produced first in the young pycnidium, to be followed later by the development of the "a" spores, hence the first tendrils consist mostly of "b" spores. Later, the two kinds of spores are found together in the same tendril, and finally, oval spores only are given off. Microtome sections have definitely shown that "a" and "b" spores can occur in the same pycnidium. The spores are given off in enormous numbers from the sporophores, and the cavity of the chamber is completely filled with abstricted spores before dehiscence occurs. It was not possible to ascertain whether each conidiophore is capable of abstricting more than one spore, or more than one kind of spore.

The ratio of length to breadth of the oval spores varies considerably according to age (Pl. XIII, fig. 1). The mature spores are wider and shorter owing to the development of two large oil drops which push out the sides and increase the width of the spore at the expense of the length. The filiform spores contain no oil drops.

The spore-tendril emerges either through a definite short-necked ostiolum at the apex of the pycnidium (Pl. XIII, fig. 11), or it may burst through the wall of the pycnidium. In the latter case the tendril is generally coarse and short as compared with the long fine tendrils, which may attain a length of 0·5 cm., given off from the ostiolum (Pl. XI, fig. 6). The tendrils, when first extruded, are whitey buff in colour, generally paler when they consist of filiform spores only; but later, when the tendrils have dried somewhat, they become yellowish in colour, and might be mistaken for the paler forms of *Cytospora* tendrils. It is difficult to say what function, if any, the filiform spores perform. Plate cultures of filiform spores gave no growth; numerous hanging drop cultures with different media were unsuccessful except in two instances. A preparation was made with acid pea-agar, 0·1 per cent. N. HCl, as the medium, and a tendril of filiform spores taken direct from the bark of Prince of Wales plum. On this medium, the filiform spores threw out lateral processes as in Pl. XIII, fig. 7, which had all the appearance of germ tubes, but unfortunately the cover slip was broken in an attempt to focus the culture under 1/12 oil immersion. Another attempt was made with the same medium; but, although spores in this case showed signs of germination, they did not advance so far as in the first instance.

There are differences of opinion as to whether these filiform bodies are really functionless spores, or merely some form of paraphyses (Reddick(15)). In stained preparations, no definite nucleus could be found in the "b" spores: the cell contents are more or less granular, and stain deeply. The absence of any definite nucleus rather points to their being paraphyses—more especially as disintegrating "b" spores can be frequently seen in the older pycnidia.

The oval "a" spores, on the other hand, germinate quite freely on a considerable number of different media, giving rise to the typical *Phomopsis* stage with "a" and "b" spores.

The strain isolated from apricot has only been found in the *Phomopsis* stage, and differs somewhat as to its morphological characters from the strains on plum and peach. The "a" spores are rather more pointed and fusoid, 7·5–9 μ × 2·5–3 μ, and "b" spores are longer and arcuate, 40–50 μ × 1.

Perithecial Stage.

When the pycnidia have opened out and are quite empty, the perithecia begin to form in the deeper-seated layers of the stroma or in the cortical tissues, either below, or by the side of the pycnidia (Pl. XIII, fig. 12), and the valsoid black necks of the perithecia mostly emerge from the same lenticel-like slits from which the pycnospores have been liberated. The base of the pycnidium is pushed up by the developing perithecia, and in its turn is burst through by the growth of the necks of the perithecia. The walls of the perithecium consist of several closely packed layers of dark thick walled mycelium, enclosing several thin delicate hymenial layers. The perithecia contain numerous asci, 50–60 μ × 7·9 μ (Pl. XIII, fig. 8), with eight two-celled ascospores, 10–17 μ × 2·5–5 μ (Pl. XIII, fig. 3). The asci are furnished with two small lobes or pores at the apex of a short neck, which can be seen very readily when stained, *intra vitam*, with a saturated solution of erythrosin in glycerine. Each cell of the bicellular ascospore contains two large oil drops.

The necks of the perithecia are long, 1–3 mm., hairy and brownish in colour when young; black and glabrous when fully mature; bent in various directions and rather irregular in shape (Pl. XI, fig. 7), with a small pale brown area at the tip. They may occur singly, or, as is more generally the case, in groups or linear series from 3–24, according to the size of the stroma and the slit in the bark. The ascospores are liberated as whitey buff globules either through the ostiolum at the apex, or from any part of the neck. It requires moist conditions for the liberation of the ascospores from the neck of the perithecium. Thus it was found difficult to obtain pure cultures of ascospores from naturally dehiscing perithecia, owing to contamination by bacteria and various rapidly growing superficial fungi. Good cultures, however, can be easily obtained, by cutting away the diseased cortical tissues round a group of perithecia, and by turning back the cut portion. The surrounding cortical tissues can then be carefully dissected away and the spherical bases of the perithecia exposed. The uninjured perithecia are then burst by slight pressure with a sterile needle and the ascospores are ejected as a mucilaginous globule, from which, with care, a fairly pure culture can be obtained. The ascospores appear to require a period of rest before germination. Ascospores obtained from old specimens which had been in the laboratory for many months germinated freely, whereas repeated attempts to germinate ascospores from freshly gathered material which had not completely dried out, failed.

Polymorphism of the organism on artificial media.

The forms of the stromata or pustules in which the pycnidia are developed vary considerably on artificial media. The more usual form of the pycnidium is more or less spherical, but aggregations of pycnidia may give rise to irregular masses, or at times the stroma may develop into a columnar or clavarieform structure (Pl. XI, fig. 4).

The same variation has been observed and fully described by the Marchals, and they point out that *Diaporthe perniciosa* shows "such a degree of polymorphism that one asks oneself if the types described under various names are not within the limits of the variability of this type."

Although not so marked, there is a certain amount of polymorphism in the fungus on the host plant, as to size and shape of stroma, number of pycnidial loculi, which vary with the size and shape of stroma; length of pycnidial and perithecial necks; variability in size and shape of pycnospores and ascospores according to age; presence or absence of "b" spores in the pycnidium, etc. It becomes more and more apparent that an adequate classification of this difficult group of fungi cannot be based on morphological characters and measurements of spores only.

PLATE CULTURES.

So far no medium has been found on which *Diaporthe* will go through both stages of its life-history satisfactorily. Platings of mass infections (*i.e.* numerous spores), and also single "a" pycnospore cultures, give rise very readily to the typical *Phomopsis* stage with both "a" and "b" spores on most of the media used; but, so far, perithecia have only developed from sowings of pycnospores in two cultures on sterile plum wood, and in one mono-pycnospore culture, isolated from a tendril with both "a" and "b" spores. Immature but normally developing perithecia were found in a sub-culture of the last-mentioned mono-pycnospore culture on prune-juice-agar 69 days old. This culture had previously developed the typical *Phomopsis* stage with both kinds of pycnospores. Black stromatic patches were found embedded in the agar beneath these patches. Another sub-culture of the same mono-pycnospore strain grown on sterilised twig of plum developed mature perithecia with typical eight-spored asci and uniseptate spores. Inoculations with this strain, and yet another pycnospore culture from a different source, have brought about typical "die back" on the current year's growth of plum, and have developed perithecia quite freely on the host plant.

On the other hand, platings of mass infections of ascospores obtained by the method above mentioned, namely, that of bursting perithecia from the under side with a sterile needle, have given rather puzzling results. Out of 11 platings taken direct from perithecia, which showed no signs of contamination, six gave the typical *Phomopsis* stage with "a" and "b" spores, and five gave pycnidia with "a" spores only. The media used for these platings were prune-juice-oat-agar, and oat-agar, but the cultures showed quite definitely that the suppression or development of "b" spores does not depend on the medium. Of the six platings which gave "b" spores, three were on prune-juice-oat-agar and three on oat-agar, and the remaining five were also grown on both media. Single ascospore cultures, however, have, so far, only produced pycnidia very slowly and sparingly. The few pustules formed appeared typical, but did not dehisce readily, and in some instances it was found necessary to open the pustules with a sterile needle in order to examine the spores; "b" spores have never been found in the mono-ascospore cultures on any medium. The "a" spores in these cultures are rather undersized and the two oil drops very pronounced, presenting much the same appearance as the older spores from tendrils on the dryer portions of the bark on the host plant.

MONO-ASCOSPORE CULTURES.

Some mono-ascospore strains produce no pycnidia, others only sparingly, and the general appearance of the cultures differs somewhat from the typical mono-pycnospore strains with "a" and "b" spores. The ascospores take nearly a week to germinate and the growth of the colony is somewhat slow. At first the surface mycelium is white, but later, on some media, such as oat-agar and oat-agar + 1 per cent. of glycerine, it becomes slightly tinged with pink. Blackened irregular patches of stromatic growth develop on the surface of the medium, forming a thin crust consisting of coarse dark mycelium with thick walls. The perithecia mostly develop in the agar beneath these stromatic patches, and the necks protrude through the superficial crust. If there are pycnidia present, some perithecia may develop in the deeper seated portions of the pycnidial stroma.

The pycnospores in such cultures are generally shorter and wider than typical pycnospores and the oil drops very pronounced. No columnar development has occurred in mono-ascospore cultures.

At first it was thought that these differences indicated the presence of two distinct fungi in close association one with the other, but judging from the results as a whole the more probable explanation is that several biologic strains exist in this variable species.

In a recent publication Ém. and Él. Marchal (38) have described a winter storage rot of apples and pears caused by a new species of *Diaporthe* named by them *D. perniciosa*, with a pycnidial stage, *Fusicoccum malorum*, which appears to be of real importance to fruit culture in the vicinities of Gembloux, Huy, Brussels, etc. Besides causing rot of stored fruits, the fungus can attack unripe fruits of plum and peach; the fruits become completely covered with stromata, dry out and fall off. Cankers also occur on the stems and branches, more especially on apple and pear, more rarely on plum and cherry. Perithecia only appear when the fruits are completely mummified, and only "capricieusement" on the bark of affected trees. The *Diaporthe* causing "die back" of plum, etc. in this country agrees very closely as to its morphological characteristics with *D. perniciosa* with the exception of the dimensions of the asci. A specimen of "die back" on plum was forwarded to Marchal for comparison with his fungus, and he is of the opinion that the two organisms are the same, and that the slight differences observed are within the limits of the variability of this species.

As far as is known to the present writer, no species of *Diaporthe* has been recorded so far as causing "die back" of stone fruit trees in this country. Until this investigation is completed, however, it is thought advisable to adopt provisionally the name of *D. perniciosa* Marchal, *f. pruni*, for the forms on peach and plum, and *Phomopsis perniciosa f. armeniaca* for the form on apricot, which, as mentioned above, shows certain morphological differences.

CULTURES OF PYCNOSPORES OF *D. PERNICIOSA*.

Cultures from tendrils containing either "a" or "a" and "b" spores grow well on various artificial media, such as prune-juice-oat-agar (made with crushed oats); plum-shoot-decoction-oat-agar, plum-shoot-decoction-agar, oat-agar, potato-agar, oat-agar + 1 per cent. glycerine, and also sterile shoots and blocks of plum wood; but less readily on potato or carrot. Large numbers of cultures isolated from different varieties of plum, apricot, peach, etc. kept under observation many months, have shown that the fungus will live a long time in pure culture.

Oat-agar. Mycelium white, felt-like, with greyish hairy pustules on the surface; submerged pustules show as black spots in the medium; medium not discoloured. In mono-pycnospore plate cultures mycelium in centre of colony raised and weft-like; pustular formation inclined to be zoned; pycnidia develop in large numbers with "a" and "b" spores; perithecia found only in one culture on this medium.

Plum-shoot-decoction-agar. Mycelium whitey-buff, fibrillose; pustules only slightly raised when single, but conglomerate pustules considerably raised above the surface of the medium, blackish, not so hairy as on oat-agar; medium discoloured reddish-brown; pycnidial development fair, not so good as on oat-agar; both kinds of spores. In mono-pycnospore cultures pycnidial formation zonal; no perithecia.

Plum-shoot-oat-agar. Mycelium white at first then turning buffish in colour, felt-like; pustules dark grey-green; colour of medium darkened. In mono-pycnospore colonies pycnidial development zonal; growth good; no perithecia.

Sterile wood. Growth sparse on heart wood; on younger wood mycelium whitish, spreading all over the wood; surface of wood blackened; pustules few, hairy, greyish-green, raised, some columnar; "a" and "b" spores; perithecia formed in two cultures.

Prune-juice-oat-agar. Mycelium loose at first, greyish; surface of medium frequently much creased; medium slightly discoloured; pustules raised, hairy, greyish-green in colour; good pycnidial development sometimes columnar; "a" and "b" spores; no perithecia.

Oat-agar + 1 per cent. glycerine. Mycelium at first white, turning pale greeny grey, rather loose; medium not discoloured; pustules raised but not columnar, hairy, greyish-green; good pycnidial development; no perithecia.

Potato. Growth poor and slow; pustules few, sometimes columnar; pycnidial development sparse.

Potato-agar. Mycelium loose, browny grey; pustules raised, hairy; medium slightly discoloured; good pycnidial development; "a" and "b" spores; no perithecia.

Sterile plum shoot. Mycelium loose, white at first, later tinged with buff; pustules hairy, raised, greyish-green; good pycnidial development; "a" and "b" spores; perithecia in one mono-pycnospore culture.

Carrot. Growth poor.

INOCULATION EXPERIMENTS.

Young Prince of Wales trees were inoculated in March and May of 1920 with various pure cultures of *Diaporthe*. A T-shaped incision, or a slit, was made in the bark as for budding, the bark raised and spores or mycelium inserted under the bark. The wound was then bound round with bast which had previously been boiled several times, and the whole covered with sheet gutta-percha, similarly treated. The hands of the operator were dipped in methylated spirit between each change of culture. The controls were treated in the same way.

The trees were inoculated on branches of the previous year's growth. So far, no typical wilt has resulted from these inoculations. The controls healed up quite healthily. The various cultures of *Diaporthe*, including the *Diaporthe* isolated from apricot, mostly showed more or less gumming and the wounds became sunken and unhealthy; inoculations with *Cytospora* isolated from Prince of Wales plum healed up quite as well as the controls. The trees have now been under observation for about 18 months.

On the other hand, the same cultures as those used for Prince of Wales plum on the young current year's growth of peach and nectarine in June, gave positive results in a comparatively short time.

(1) *Phomopsis* stage of *Diaporthe* isolated from bark of Prince of Wales on prune-juice-oat-agar 71 days old, produced rapid wilt on nectarine "Elruge" in 25 days (Pl. XIV, fig. 1 (2)); two inoculations, both positive.

(2) *Phomopsis* stage of *Diaporthe* isolated from bark of Coe's Golden Drop on oat-agar, 32 days old; rapid wilt on peach (var. unknown) in 25 days (Pl. XIV, fig. 1 (4)); three inoculations all positive.

(3) *Phomopsis* stage of *Diaporthe* isolated from bark of apricot on prune-juice-oat-agar, 21 days old; two inoculations with spores, on peach (var. unknown) positive in 64 days (Pl. XIV, fig. 2 (1)); and a third with mycelium as inoculum, positive after one year and 24 days.

(4) *Phomopsis* stage of *Diaporthe* isolated from bark of peach (var. unknown) on prune-juice-oat-agar, 44 days old, wilt in 17 days; two inoculations, both positive.

(5) *Phomopsis* stage of *Diaporthe* isolated from the bark of apricot on prune-juice-oat-agar, 52 days old; on young current year's growth of apricot, var. Breda; one inoculation positive after about one year.

(6) *Phomopsis* stage of *Diaporthe* isolated from the bark of Prince of Wales plum, on prune-juice-oat-agar, 73 days old; on young current year's growth of apricot, var. Kaisha; one inoculation positive after ten months.

(7) *Phomopsis* stage of mono-pycnospore culture of *Diaporthe* isolated from bark of Prince of Wales plum, on oat-agar, 18 days old (mycelium used as inoculum); on Jefferson plum; one inoculation positive after 61 days. (N.B. This mono-pycnospore culture produced perithecia on the host plant in three months.)

(8) Same culture as in (7) on young current year's growth on peach seedling (Royal George selfed); one inoculation positive; shoot dead after 11 months.

The above results show that the period which elapses between infection and the onset of the disease is very variable, and probably depends more upon the age and succulence of the parts infected, than the age of the cultures used.

Young peach shoots are more succulent than either plum or apricot. The above inoculations on the current season's growth of the various hosts were made during the months of June and July.

The organism was re-isolated from the tissues above and below the point of inoculation in at least one specimen of each of the above cases. These results prove quite definitely that all the above strains of *Diaporthe* are parasitic and cause "die back" on peach, nectarine, apricot and plum. The failure to bring about wilt on Prince of Wales plum is probably due to the inoculations having been made on older wood, and the organism has not yet permeated the tissues sufficiently to cause wilt.

Unripe apples of the varieties Lord Suffield, Peasgood Nonsuch and Bramley Seedling (Pl. XII, fig. 4) were inoculated through an incision in the skin, with mono-ascospore and mono-pycnospore cultures of *Diaporthe* isolated by the author from "die back" on Prince of Wales plum and Victoria seedling, and with a monospore culture of *D. perniciosa* forwarded by Prof. Marchal. Mycelium was used for the inocula in the case of Marchal's culture and the mono-ascospore cultures, and spores in the case of the pycnospore cultures. Decided patches of rot, identical in appearance, resulted in a few days from inoculations with plum ascospore and Marchal's cultures on Lord Suffield apples, and by plum pycnospore and Marchal's cultures on Peasgood Nonsuch. Bramley Seedling, a later apple, which was consequently still very hard and green, proved more resistant, and 14 days after inoculation only showed slight traces of rot.

Inoculations were also made on fruits of Victoria plum (Pl. XII, fig. 5) in various stages of ripeness from green fruits to those showing colour, with Marchal's culture and various other mono-ascospore and mono-pycnospore cultures. Marchal's culture proved to be the most virulent, giving rise in a few days to dark patches of rot $\frac{1}{2}$–$\frac{3}{4}$ inch in diameter, on all the fruits inoculated. The patches of rot caused by the author's cultures appeared somewhat paler in colour. When pycnospores were used as inoculum the rot did not develop so rapidly, probably owing to the time required for the germination of the spores.

All the controls remained healthy.

The recent investigations of Wiltshire(59) as to the causes of "die back" have given interesting results. He describes the presence of bacterial masses in the diseased cortex together with fungal hyphae, the latter, in some instances developing cytospora-like fructifications, and suggests that there may be some relationship between the two organisms.

So far he has been unable to grow the bacteria on artificial media, and gives no description of the accompanying fungus. But it is quite possible that his work may throw light on the problem of the parasitism of *Cytospora*, and may show that concomitant infection with other organisms may be necessary in order to enable *Cytospora* to develop as a parasite in the host tissues.

The present writer has also observed rod-shaped bacteria in the disorganised cortical tissues attacked by *Diaporthe*; but, seeing that inoculations with *D. perniciosa* in pure culture have given definite results, more especially on peach, that they have caused considerable disturbance in one year old wood of plum, and also that they have set up rot in unripe apple and plum fruits, no concomitant organism appears to be necessary in order to bring about infection with *D. perniciosa.*

CONTROL MEASURES.

In spite of so little being known as yet as to the conditions which are conducive to infection of stone fruit trees by the fungi described in this paper, a few suggestions for control measures can be made.

Good cultural conditions and careful pruning are of prime importance. These fungi are all believed to be wound parasites and hence all cut surfaces should be treated with styptic or some antiseptic dressing. Very susceptible varieties, such as Prince of Wales plum, should be budded rather than grafted, and, if possible, well above soil level. In the case of standards the stock should be taken up to form the stem of the tree, and budded or grafted at the height required. The buds should not be taken from young, but from older trees, which have survived for a considerable number of years.

Care should be taken to examine the internal tissues of the shoots from which the buds are taken, and any shoot showing any traces of internal discolouration should be rejected.

When the bud has grown, the portion of the stock above the bud should be cut away and not left to act as a support for the growing shoot, as is so often the practice in nurseries; the cut surface should be pared smooth and treated with styptic. The same precautions hold good in the case of grafting, and the internal tissues of the stock also should be above suspicion.

Nothing is known as yet as to whether the variety of stock has any influence on the resistance or susceptibility of the scion. The stocks should be grafted young in order to avoid any great disparity in size between stock and scion, and to enable the wound to callus over completely.

The examination of a considerable number of young standard trees attacked by *D. perniciosa* has shown that in the majority of cases infection has occurred in the region of the graft, or a large pruning scar which has not healed over completely.

As regards the treatment of older trees, all diseased or dead parts should be carefully pruned back to healthy wood and the pruning scars treated as advised above. In damp weather the various fungi sporulate freely on dead or dying wood, hence all refuse and prunings should be burnt, and not left lying on the ground to infect the soil and the surrounding trees.

Implements used for cutting away diseased portions should be disinfected before use on healthy trees, more especially after a spell of wet weather. Saws, knives, the hands of the operator, etc. can be dipped in a 3 per cent. watery solution of lysol, but the lysol should on no account be used for treating pruning scars.

Specimens of mature pycnidia have been found on the host plants from January to September, and perithecia develop promiscuously throughout the year. The numbers of perithecia found are few as compared with the excessive development of pycnidia on the affected trees. This suggests that only certain strains of pycnospores are capable of forming perithecia and that the pycnidial stage is the parasitic stage. If spraying is resorted to, it should be done throughout the summer after rain; but this is hardly to be recommended on the score of expense.

In conclusion I wish to express my thanks to Mr Lobjoit, Controller of Horticulture, and Mr E. W. Lobjoit for bringing the disease to my notice and for giving me valuable information and every facility for collecting material and inspecting trees; to Mr A. D. Cotton and Mrs Alcock of the Pathological Laboratory of the Ministry of Agriculture for their help in supplying me with specimens and information as to the incidence of "die backs" of fruit trees in various parts of the country; to Miss E. Gunnell, Horticultural Superintendent of Devon County Agricultural Committee, for supplying me with diseased specimens; to Prof. Marchal and Mr W. B. Grove for help in identifying the fungi described; to Mr Crane of this Institution; to Mr T. Richardson, County Inspector for Berks., and lastly to A. F. Emarton, laboratory assistant, for valuable help in making numerous microtome preparations and for taking the photographs for the Plates.

SUMMARY.

1. *Diaporthe perniciosa*, first isolated by Marchal from rots in stored fruits, has proved to be parasitic and one of the causes of "die back" in stone fruit trees.

2. The various strains of *D. perniciosa* isolated from "die back" on peach and different varieties of plums have proved to be parasitic, morphologically identical, and cause definite wilt and "die back" on peach, plum, apricot and nectarine. Cross inoculations of the same organisms on two year old wood of Prince of Wales plum have so far not brought about typical symptoms of "die back," but considerable disturbance, accompanied by gumming, has occurred at the points of inoculation. A strain obtained from apricot, which shows morphological differences, has also been proved to be capable of causing "die back" on peach and apricot. All the controls remained healthy.

3. The progress of *D. perniciosa* in the host tissue is slow; infection occurs a considerable time, in some cases years, before any external symptoms appear. The mycelium permeates the internal tissues more especially the medullary rays, cortical tissues, phloem cambium, and vessels.

4. *D. perniciosa* has a pycnidial or *Phomopsis* stage with "a" and "b" spores, and a perithecial or *Valsa* stage with numerous eight-spored asci. The pycnospores emerge as whitish tendrils or globules through numerous lenticel-like slits in the bark; the perithecial necks are long and protrude mostly through the same slits as the tendrils, and the ascospores are given off either through the ostiolum at the tip of the neck of the perithecium, or may burst through any part of the neck.

5. The external symptoms are sunken areas and longitudinal slits in the bark; elongated strands of numerous lenticel-like transverse slits through which tendrils and perithecial necks emerge; rapid wilt and browning of the trees in summer, or premature yellowing and fall of the leaves in the autumn.

6. The internal tissues are discoloured and secondary thickening arrested.

7. The pycnidial stage occurs on living or more or less moribund bark, the perithecial stage on the parts which are dead.

8. *D. perniciosa* shows a certain degree of polymorphism on the host plant; on artificial media this polymorphism is much more marked.

9. The *Phomopsis* stage of *D. perniciosa* grows and sporulates well on artificial media, but perithecia develop sparingly in cultures from pycnospores.

10. Cultures of mass infections of ascospores of *D. perniciosa* have given rise in some cases to the *Phomopsis* stage with "a" and "b" spores; in others no "b" spores could be found. Mono-ascospore cultures develop the pycnidial stage very sparingly, and no "b" spores have been observed in any of the cultures. Large numbers of perithecia develop in mono-ascospore cultures on various media.

BIBLIOGRAPHY.

(1) Aderhold, R. (1903). Über das Kirschbaumsterben am Rhein, seine Ursachen und seine Bekämpfung. *Arb. d. Biol. Abt. f. Land- und Forst-Wirthschaft am. kais. Gesundheitsamt*, Bd III, Heft 4.

(2) Appel, O. (1904). *Naturw. Zeitschr. f. Land- und Forst-Wirtschaft*, p. 313.

(3) Bailhache, C. H. (1920). *The Fruit Grower*, Nov. 25th.

(4) Belgrave, W. N. C. (1915). On Diseases of Plum Trees caused by some species of *Cytospora*. *Ann. Appl. Biol.* II, Nos. 2 and 3, July.

(5) Cooke, M. (1901–1902). *Journ. Roy. Hort. Soc.* XXVI.

(6) —— (1902). *Ibid.* XXVII.

(7) —— . *Fungoid Pests of Cultivated Plants.*

(8) Clinton, G. P. (1911–1912). Chestnut bark disease. *Conn. Agri. Exp. Stat. Report* (with good bibliography).

(9) Cotton, A. D. (1902). *Journ. Roy. Hort. Soc.* XXVII, 936.

(10) Darnell-Smith, G. P. (1918). An account of some observations upon the life history of *Phoma citricarpa*. *Proc. Linnean Soc. N.S. Wales*, 43.

(11) Delacroix, G. (1905). Sur une Maladie des Armandiers de Provence. *Bul. Trimestre Soc. Myc. de France*, XXI.

(12) Diedicke, H. (1911). Die Gattung *Phomopsis* und *Plenodesmus*. Preuss. *Ann. Myc.* IX.

(13) Dowson, W. J. (1913). On a Disease of Greengage Trees caused by *Dermatella prunastri*. *New Phytologist*, XII, No. 6.

(14) Ewert, R. (1908). Ist das Rheinische Kirschsterben auf eine Pilzkrankheit zurück zu führen. *Proskauer Obstbauzeitung*, Jahrg. 13.

(15) Farlow, W. G. (1912). The Fungus of the Chestnut Blight. *Science*, N.S., May.

(16) Frank, A. B. (1899). *Deutsche Landw. Presse*, No. 83.

(17) Goethe, R. (1899). Das Absterben der Kirschenbaüme in den Kreisen St Goar, St Goarhausen und Unterlahn. *Ibid.* No. 99.

(18) Grove, W. B. (1917). The British Species of *Phomopsis*. *Kew Bull.* No. 2.

(19) —— (1919). Species placed by Saccardo in the genus *Phoma*. *Ibid.* No. 4.

(20) HAHN, G. G., HARTLEY, C. and PIERCE, R. G. (1917). A nursery blight of cedars. *Journ. Agric. Res.* x.

(21) ——— ——— ——— (1920). *Phomopsis Juniperovora. Phytopath.* x, No. 4.

(22) HARTER, L. L. (1913). Foot rot of Sweet Potato. *Journ. Agric. Res.* I, No. 3.

(23) ——— (1914). Fruit rot, leaf spot and stem blight of Egg Plant, caused by *Phomopsis vexans. Ibid.* II, No. 5.

(24) HARTER, L. L. and FIELD, E. C. (1912). *Diaporthe,* the ascogenous form of Sweet Potato Dry Rot. *Phytopath.* II, No. 3.

(25) ——— ——— (1913). Dry Rot of Sweet Potatoes caused by *Diaporthe batatis. U.S. Dep. Agric. Bur. Pl. Ind., Bull.* 281.

(26) ——— (1917). Pod blight of Lima Bean caused by *Diaporthe phaseolorum. Journ. Agric. Res.* XI, No. 10.

(27) HEALD, F. D. and GARDNER, M. W. (1913). The relative prevalence of pycnospores and ascospores of the Chestnut blight fungus during the winter. *Phytopath.* III, No. 6.

(28) HEALD, F. D. and STUDHALTER, R. A. (1914). Birds as carriers of the Chestnut Blight Fungus. *Journ. Agric. Res.* II, No. 6.

(29) HOEHNEL, VON (1906). *Fragmente zur Mykologie,* No. 87.

(30) HUBERT, E. E. (1920). Observations on *Cytospora chrysosperma* in the Northwest. *Phytopath.* x, No. 10.

(31) KEEPER, W. E. (1914). Pathological Histology of the *Endothia* canker of Chestnut. *Ibid.* IV, No. 3.

(32) LAUBERT, R. (1904). Zur Morphologie einer neuer *Cytospora. Cent. f. Bakt.* II, Bd. XII.

(33) LEWIS, C. E. (1909). Apple diseases caused by *Coryneum follicolum* and *Phoma mali. Maine Agric. Exp. Stat. Bull.* 170.

(34) LÜSTNER, G. (1907). Untersuchungen über die Ursache des Rheinischen Kirschbaumsterbens. *Ber. d. Königl. Lehranstalt für Wein-, Obst-, und Gartenbau zu Geisenheim am Rh.*

(35) MASSEE, G. (1902). Disease of Nursery Stock. *Gard. Chron.* Sep. 27th.

(36) ——— (1902). *Journ. Bd. Agric.* IX.

(37) ——— (1901–1902). *Journ. Roy. Hort. Soc.* XXVI.

(38) MARCHAL, ÉL ET ÉM. (1921). Contribution à l'étude des Champignons Fructicoles de Belgique. *Bull. d. l. Soc. Roy. de Bot. de Belgique,* T. LIV. N.S. IV.

(39) MURRILL, W. A. (1906). A serious Chestnut disease. *Journ. N.Y. Bot. Gardens,* No. 7.

(40) ——— (1906). Further remarks on a serious chestnut disease. *Ibid.* No. 7.

(41) ——— . *Torreya,* v, 6, No. 9.

(42) ——— . *Ibid.* v, 8, No. 5.

(43) NIETSCHE, T. *Pyrenomycetes Germanica.* Valsaceae.

(44) POTEBNIA, A. (1907). *Fusicoccum pruni. Ann. Myc.* v.

(45) REDDICK, D. (1909). Necrosis of grape vine. *Conn. Univ. Agric. Exp. Stat. Dep. Pl. Path. Bull.* 263.

(46) REHM, H. (1910). *Eutypella prunastri,* f. Crataegi. *Ann. Myc.* VIII.

(47) ROBERTS, J. W. (1913). Rough bark disease of Yellow Newtown Apple. *U.S. Dep. Agric. Bur. Pl. Ind., Bull.* 280.

(48) ROLFS, F. M. (1910). Winter killing of twigs, cankers and sun-scald of peaches. *Missouri St. Fruit Exp. Stat., Bull.* 17.

(49) RUE, C. DE LA. (1920). Isolating single spores. *Bot. Gaz.* LXX, No. 4, Oct.

(50) RUHLAND, W. (1904). Ein neuer, verderblicher Schädling der Eiche. *Cent. f. Bakt.* Abt. 2, Bd. XII.

(51) SALMON, E. S. (1910). *Journ. S.E. Agric. Coll. Kent,* No. 19.

(52) Shear, C. L. (1917). End rot of Cranberries. *Fusicoccum putrefaciens. Journ. Agric. Res.* xi, No. 2.

(53) —— (1911). The ascogenous form of fungus causing dead arm of Grape. *Phytopath.* i, Nos. 3 and 4.

(54) Shear, C. L. and Wood, A. K. Studies of fungus parasites belonging to the genus *Glomerella. U.S. Dep. Agric. Bur. Pl. Ind., Bull.* 252.

(55) Sorauer, P. (1900). Das Kirschbaumsterben am Rh. *Deutsch. Landw. Presse,* No. 18.

(56) Wehmer, C. (1898). Zum Kirschbaumsterben am Rh. *Ibid.* No. 96.

(57) —— (1894). Über das massenhafte Vorkommen eines Kernpilzes auf den Alleebäumen etc. *Jahresber. d. Naturhis. Gesell.* Hanover.

(58) Wilson, M. (1919–1920). A new species of *Phomopsis* parasitic on the Douglas Fir. *Trans. and Proc. Bot. Soc. Edinburgh,* xxviii, Pt I.

(59) Wiltshire, S. P. (1920). A bacterial infection of plum trees. *The Annual Report, Long Ashton Exp. Stat. Bristol.*

(60) Woburn (1900). *Exp. Fruit Farm, Rept.* ii.

(61) Wolf, F. A. and Stanford, E. E. (1918). A *Macrophoma* disease of Figs. *Phytopath.* viii, No. 1.

(62) Wormald, H. (1912). *Cytospora* Disease of Cherry. *Journ. S.E. Agric. Coll. Wye, Kent,* No. 21.

EXPLANATION OF PLATES XI—XIV

PLATE XI.

Fig. 1. (1) Healthy stem. Prince of Wales plum. $\times \frac{3}{4}$. (2) *Phomopsis* stage of *Diaporthe perniciosa* showing tendrils of spores on Prince of Wales plum. (3) *Phomopsis* stage of *D. perniciosa* on stem of standard of seedling of Victoria plum.

Fig. 2. Canker on stem of peach seedling due to *Cytospora,* probably *C. leucostoma* var. *cincta.*

Fig. 3. Branch of seedling from Victoria plum, attacked by *D. perniciosa,* showing discolouration of internal tissues in (*b*) and (*c*); (*a*) healthy branch.

Fig. 4. Slope culture of *Phomopsis* stage of *D. perniciosa* isolated from peach, on potato + 1 % glycerine; 143 days old, showing columnar development of pycnidia. $\times \frac{2}{3}$.

Fig. 5. Sections of diseased branches of Prince of Wales plum attacked by *D. perniciosa,* showing arrested secondary thickening and discolouration of internal tissues.

Fig. 6. Tendrils of *Phomopsis* stage of *D. perniciosa* showing coarse and fine tendrils. × 6.

Fig. 7. Perithecia of *D. perniciosa* on bark of Pershore plum. × 3.

PLATE XII.

Fig. 1. Young tree of Prince of Wales plum attacked by *D. perniciosa* in the region of the graft. Pustules showing on the bark in the diseased area d–d_1.

Fig. 2. The reverse side of the same tree showing incomplete healing over of the graft wound.

Fig. 3. (*a*) Pycnospore culture of *D. perniciosa* isolated from peach on prune-juice-oat-agar, 24 days old. (*b*) Pycnospore culture of *D. perniciosa* isolated from Prince of Wales plum on prune-juice-oat-agar, 44 days old.

Fig. 4. Inoculations of apple fruits with *D. perniciosa* from various sources. (1) Peasgood Nonsuch; control. (2) Peasgood Nonsuch inoculated with mono-pycnospore culture from bark of Prince of Wales plum. (3) Lord Suffield inoculated with mono-ascospore culture isolated from bark of Prince of Wales plum. (4) Bramley Seedling inoculated with same culture as (2). (5) Bramley Seedling control. In (2) and (4) pycnospores were used as inoculum, in (3) mycelium.

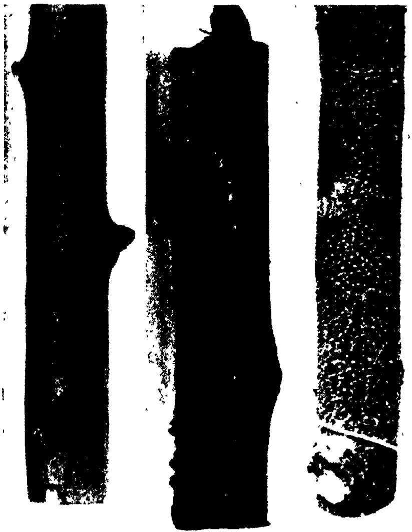
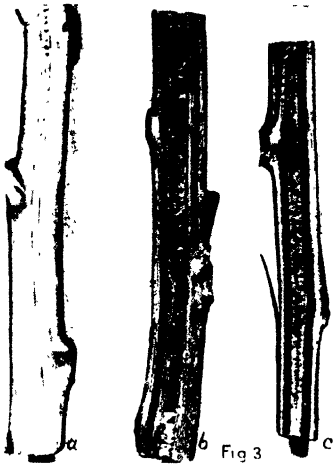


Fig. 1



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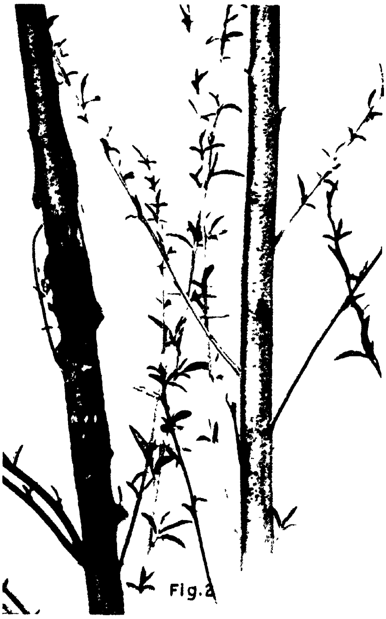

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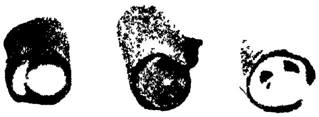

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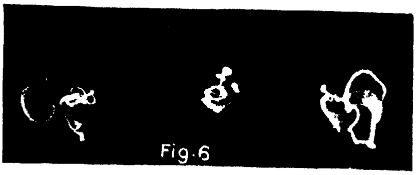

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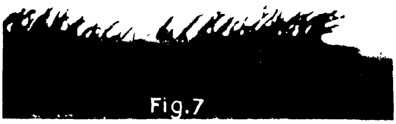


Fig. 7

CAYLEY.—Fungi associated with "Die Back" in Stone Fruit Trees. I. (pp. 253—275.)

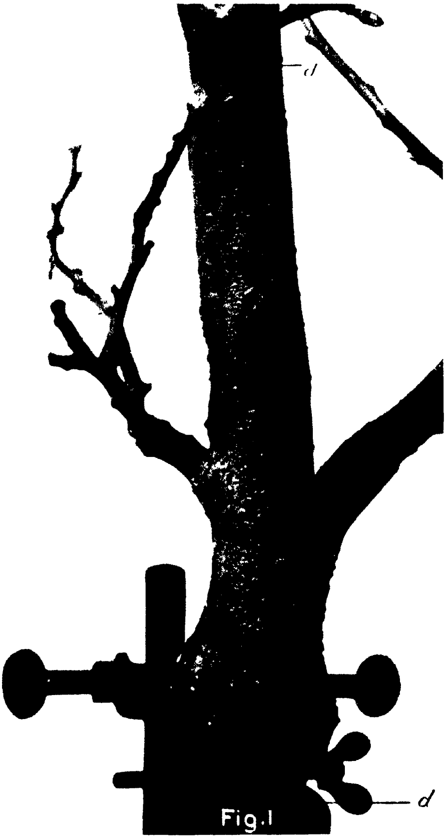


Fig. 1

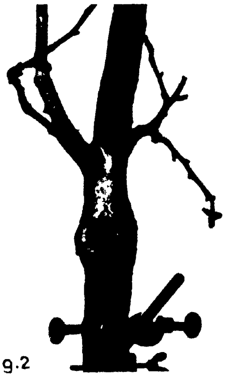
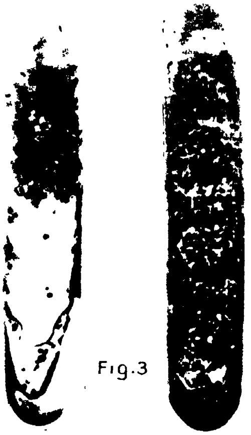


Fig. 2



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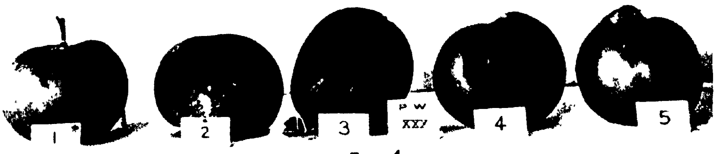
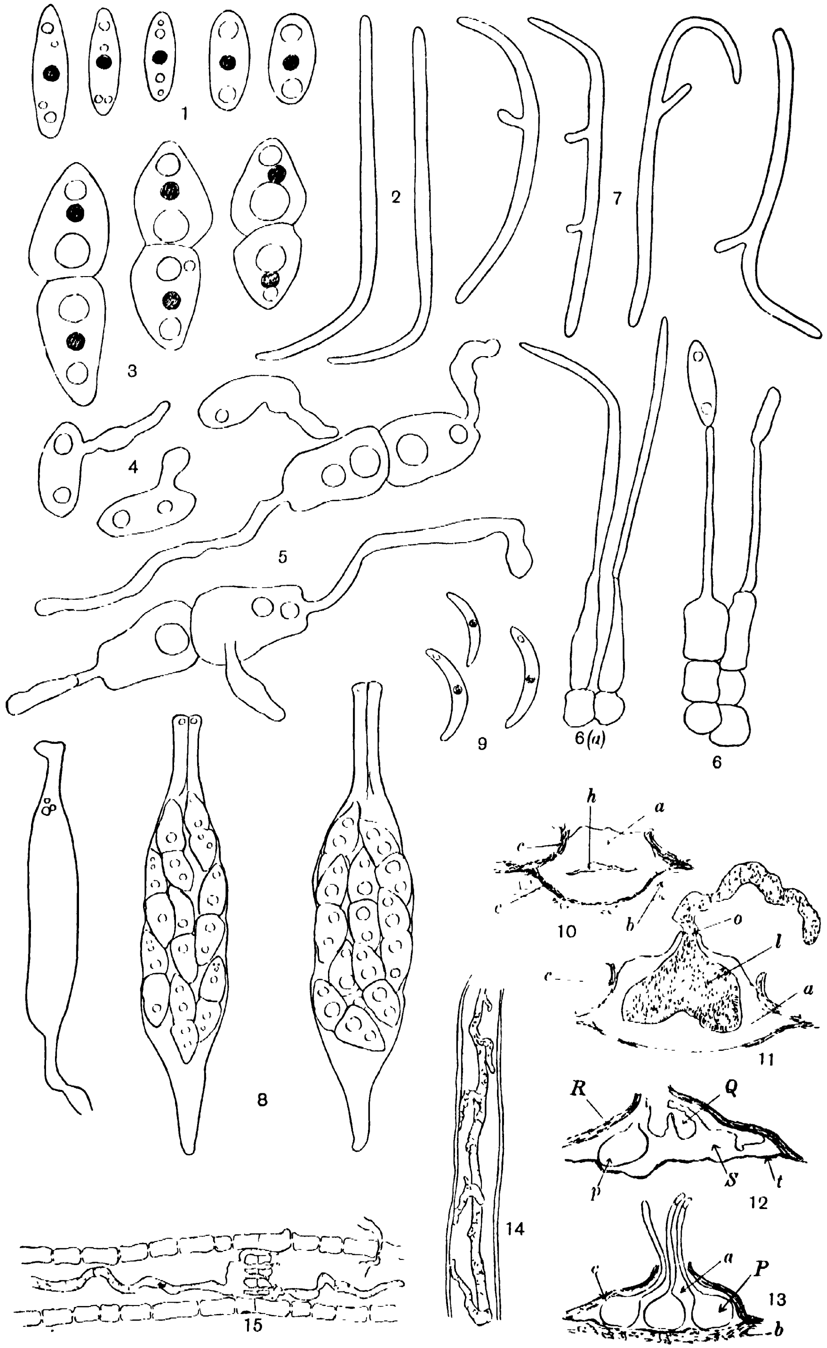


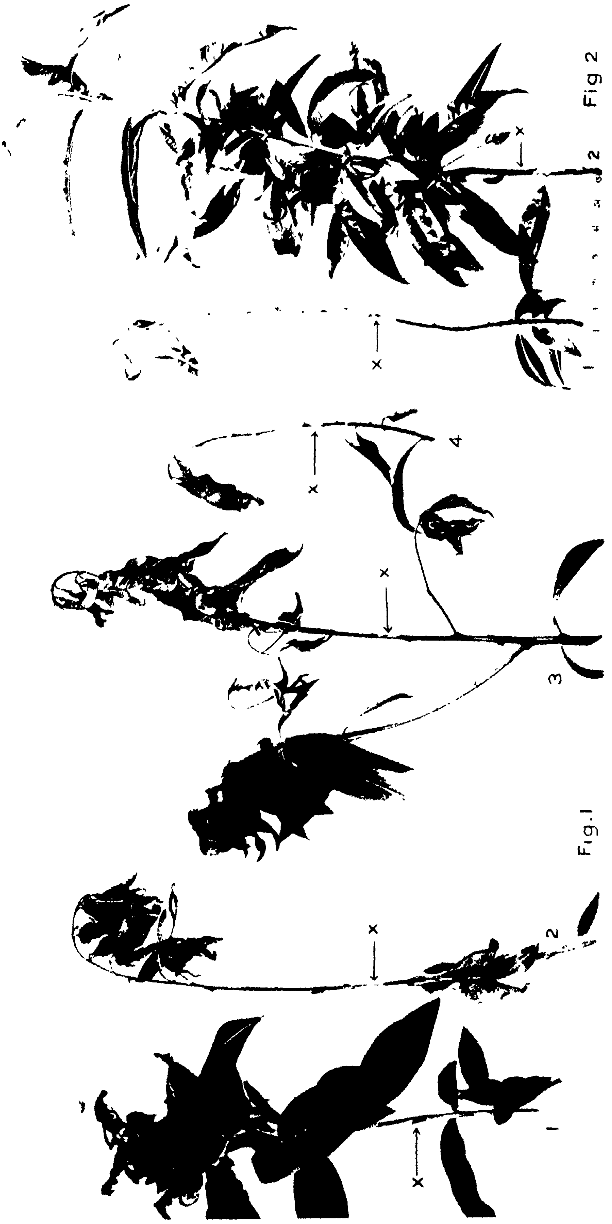
Fig 4

CAYLEY.—Fungi associated with "Die Back" in Stone Fruit Trees. I. (pp. 253—275.)



CAYLEY.—Fungi associated with "Die Back" in Stone Fruit Trees. I. (pp. 253—275.)

THE ANNALS OF APPLIED BIOLOGY. VOL. X, NO. 2 PLATE XIV



CAYLEY.—Fungi associated with "Die Back" in Stone Fruit Trees. I. (pp. 253—275.)

Fig. 5. Inoculation of Victoria plum fruits of varying degrees of ripeness, with *D. perniciosa* from various sources. (1) Control; ripe plum. (2) Ripe plum inoculated with multi-sporous pycnospore culture isolated from bark of Victoria plum (3) Unripe plum inoculated with mono-pycnospore culture isolated from bark of Victoria plum. (4) Unripe plum inoculated with mono-ascospore culture isolated from bark of Prince of Wales plum. (5) Unripe plum inoculated with Marchal's mono-spore culture. In (3) pycnospores used as inoculum, in (2), (4), (5), mycelium.

PLATE XIII.

Fig. 1. "a" pycnospores of different ages. *D. perniciosa.* x 2000.

Fig. 2. "b" pycnospores. *D. perniciosa.* x 2000.

Fig. 3. Ascospores. *D. perniciosa.* x 2000.

Fig. 4. Germinating "a" pycnospores. *D. perniciosa.* x 2000.

Fig. 5. Germinating ascospores. *D. perniciosa.* x 2000.

Fig. 6. Sporophores and young "a" pycnospores. *D. perniciosa.* x 2000.

Fig. 6 *a*. Sporophores and young "b" pycnospores. *D. perniciosa.* x 2000.

Fig. 7. Germinating (?) "b" pycnospores. *D. perniciosa.* x 2000.

Fig. 8. Asci of different ages. *D. perniciosa.* x 1000.

Fig. 9. Pycnospores of *Cytospora.* x 2000.

Figs. 10, 11. Development of pycnidia in the sorus, *D. perniciosa.* x 20. (*a*) mycelial tissue of the sorus; (*b*) disorganised tissues of the cortex of the host; (*c*) corky layers of the bark; (*h*) proliferous layer of the young pycnidium; (*l*) loculus of the pycnidium; (*o*) ostiolum.

Fig. 12. Stroma of *D. perniciosa.* x 20. (*p*) young perithecium; (*Q*) disorganised chamber of empty pycnidium; (*R*) cork; (*S*) mycelial tissue of stroma; (*t*) black delimiting line of the stroma.

Fig. 13. Group of perithecia of *D. perniciosa*: (same figuring as in Figs. 10, 11).

Fig. 14. Mycelium of *D. perniciosa* passing vertically up a fibre.

Fig. 15. Mycelium of *D. perniciosa* passing horizontally through the pits of a medullary ray cell.

PLATE XIV.

Fig. 1. Inoculation on peach and nectarine. (1) Control (attacked by aphis). (2) Nectarine "Elruge" inoculated under the bark with pycnospores of *D. perniciosa* isolated from Prince of Wales plum; 26 days after inoculation. (3) Same as (2). (4) Peach (var. unknown) inoculated with pycnospores of *D. perniciosa* isolated from Coe's Golden Drop plum.

Fig. 2. (1) Peach (var. unknown) inoculated with pycnospores of *D. perniciosa*, isolated from apricot; 64 days after inoculation. (2) Control. x = point of inoculation.

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ON A NEW SPECIES OF MONIEZIA FROM THE SHEEP, *OVIS ARIES*

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University College of Wales, Aberystwyth.

(With Plate XV and 2 Text-figures.)

THE present paper represents a part of certain work which I have been carrying out upon tape worms of the genus Moniezia, with the aid of a special research grant from the Ministry of Agriculture and Fisheries, and a supplementary research exhibition from the Honble. Samuel Vestey, to both of whom I would like to take this opportunity of tendering my thanks.

The material was collected, together with other species of Moniezia, from the small intestines of sheep killed at the Municipal Slaughterhouse, Aberystwyth.

GENERAL APPEARANCE.

When fresh, the strobilia is extremely translucent, the anterior portion is clear and colourless, the posterior portion, due to the egg content of the gravid uteri, is dotted with yellow. The line of the ventral excretory canals is easily visible to the naked eye, as are the genital papillae.

SCOLEX.

The scolex is small, pear-shaped, and almost square *en face.* It varies in length from ·625 mm. to 1·25 mm., and in breadth from ·496 mm. to ·750 mm. Its distinguishing feature is, however, that in any individual scolex, the length always exceeds the breadth. The scolex tapers gradually towards the neck, from which it is not clearly separated. The suckers are situated anteriorly at the four corners. They are only slightly raised from the main surface level of the scolex, and are not distinctly separated from it. The average width of the suckers is ·250 mm. The sucker openings are oval in shape, and inclined diagonally forwards; their average breadth is ·04 mm., and depth ·06 mm. The sucker wall is exceedingly muscular and distinctly separated from the undifferentiated scolex parenchyma. It consists of a ground tissue of parenchyma composed of well defined cells, and bounded by a strongly developed layer of circular muscle, and an outer, less well developed layer of longitudinal muscle. The ground tissue is very thickly interspersed with longitudinal, transverse and oblique muscles.

NECK.

The neck is long and slender, not distinctly separated from the scolex. It varies in length from 2·38 mm. to 4·63 mm., and in breadth from ·214 mm. to ·496 mm.

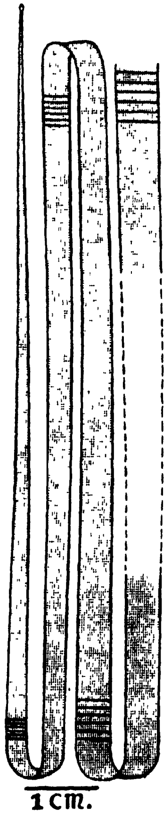
STROBILA.

In common with those of the other members of the genus, the segments are always much broader than long, the terminal segments having a tendency to become longer and narrower. The strobila rapidly increases in width. At 10 cms. from the scolex, the average width of the segments is 2·75 mm., and their average length ·458 mm.; at this point there is no visible sign of reproductive organs. At 20 cms. from the scolex, the segments average 3·75 mm. in width, and ·81 mm. in length; the genital anlage is visible, and has the pistol shape, characteristic of the genus; the genital pores have not pierced the edge of the segment; the testes are visible. At 30 cms. from the scolex, the average breadth of the segments is 4·66 mm., and their average length 1·0 mm.; the reproductive organs are now clearly visible, and the genital pores have opened to the exterior, the genital papillae being clear and distinct to the naked eye.

The longest specimen obtained measured 185 cms. in length, the end segment, some segments evidently having become detached, being 9 mm. wide. The greatest width of segments observed was 12 mm. and greatest length 3 mm.

HISTOLOGY.

The general histology of the species is typical of the genus, and calls for little comment. The muscular system is not well developed, particularly the circular muscle layer, whilst the constituent longitudinal muscle fibres in the older segments lose much of the



Text-fig. 1. Strobila of *M. translucida.* To show the rate of increase in the width of the strobila and in the length of the segments.

aggregation into little bundles which is typical of the younger segments. The external cuticular layer is thin.

INTERPROGLOTTIDAL GLANDS.

The interproglottidal glands are linear. They are short, but well defined, varying in length from ·9 mm. to 1·6 mm. The length of the glands depends to some extent upon the width of the segments, but not wholly so. Thus, glands 1 mm. long have been observed in segments 3·6 mm. wide, and in segments 8·8 mm. wide, whilst in other segments 8 mm. wide, glands 1·6 mm. long have been observed.

EXCRETORY SYSTEM.

In the strobila the lumen of the dorsal canal is small, varying in diameter from ·004 mm. to ·008 mm. Its course is very irregular, it usually lies on the median side of the ventral canal, and slightly dorsal to it. It may, however, lie in the same dorso-ventral line as the ventral canal, or may even lie ventral to the centre point of the latter. Except in the scolex, the dorsal canal has not been observed to lie external to the ventral canal.

The cuticular lining of the dorsal canal is much thicker than that of the ventral canal, and contains darkly staining bodies resembling very small nuclei. The dorsal canal is surrounded by a well defined layer of round or oval cells, considerably larger than those of the surrounding parenchyma, and possessing large and well defined nuclei. The layer is usually one cell thick, sometimes two.

The lumen of the ventral canal varies in diameter from ·04 mm. to ·06 mm. At the posterior end of each segment, the ventral canals are connected by a transverse commissure of ·02 mm. diameter. The cuticular lining of the ventral canal is thin. The canal is surrounded by an ill-defined layer of flattened ovoid cells, with fairly well-defined nuclei.

In the unsegmented neck region, the dorsal and ventral canals are more nearly equal in diameter, ·008 mm. and ·012 mm. respectively. The dorsal canals lie slightly internal to the ventral canals, which are not connected by transverse commissures in this region.

In the scolex the canals are of the same dimensions as in the neck. At a point approximately in a line with the centre of the suckers, the ventral canals are connected by a transverse commissure; ·08 mm. anteriorly, the dorsal canal of each side unites with its ventral canal and ·02 mm. anterior to this union, the joint canals unite in the middle line.

In many instances it was noticed that the ventral canals gave off large longitudinal branches which ran through three or four segments and then joined up again with the parent canal, whilst in one scolex, the dorsal and ventral canals of one side only were united by a short commissure. The number and topography of the excretory canals in this species, therefore, would appear to be liable to slight variation.

In the original terminal segment of the strobila, the dorsal canal of each side unites with its ventral canal and the two joint canals open by a common pore in the mid-ventral line.

NERVOUS SYSTEM.

Identification of the nervous system presented difficulties, no entirely satisfactory method of differential staining having been discovered. It has been possible to determine, however, that the nervous system is of the usual type. The cephalic ganglion is composed of numbers of transverse fibres massed together to form a nerve bundle, which runs across the scolex, passing between the dorsal and ventral excretory canals, immediately posterior to the fusion of the dorsal and ventral canals of each side as described above. The cephalic ganglion gives off nerve fibres to the four suckers, and a pair of main lateral longitudinal nerves. These latter run, one on each side, the whole length of the strobila, external to the excretory canals, crossing the genital ducts ventrally.

REPRODUCTIVE SYSTEM.

The genital papillae are easily visible to the naked eye, they are usually situated in the anterior third, and invariably in the anterior half of the lateral borders of the segment.

Cirrus and vagina open into the common genital papilla, on the right side of the segment the cirrus being dorsal to the vagina, whilst on the left-hand side, the cirrus is ventral to the vagina.

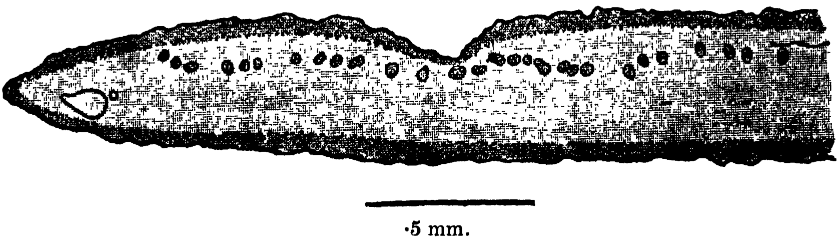
The antero-posterior relations of cirrus and vagina are not quite constant; on the right side of the segment the cirrus is usually anterior to the vagina, and on the left side, posterior to it. This condition occurs in about 75 per cent. of the segments. In the remaining 25 per cent. of the segments, cirrus and vagina of either side may lie in the same lateral plane, or, the vagina of the right side may be anterior, whilst the vagina of the left side may be posterior. The genital canals invariably cross both excretory canals and longitudinal nerve, dorsally.

MALE ORGANS.

The cirrus pouch runs straight inward towards the median line, it averages ·12 mm. long and ·06 mm. broad. The expanded cirrus is ·14 mm. long, convolutions of the cirrus in the cirrus sac being uncommon.

The lumen of the cirrus at its lateral end averages ·004 mm. in diameter, widening gradually to ·02 mm. in diameter at its median end, where it constricts suddenly to ·004 mm. in diameter to leave the cirrus pouch. Immediately after leaving the pouch it dilates to ·04 mm. diameter to form the vas deferens, which, on the left side of the segment, at once crosses to the dorsal side of the vagina. The vas deferens performs numerous convolutions, whilst running inwards and downwards, crossing the ovary dorsally.

The testes are uniformly distributed throughout the median field in both young and old segments, and are confined to the posterior portion of the segment, not being found anterior to a plane passing through the anterior border of the shell gland. They are thus arranged in a long, narrow, rectangle. As regards their dorso-ventral distribution, the testes are invariably confined to a single dorsal layer. No testes were found in the lateral fields, that is, external to the excretory canals. In a mature segment, about 300 testes are visible.



Text-fig. 2. T.S. of left half of a proglottid to show the distribution of the testes.

The cirrus pouch is a very muscular organ, consisting of the following tissue layers in order from within outwards:

1. *Lumen.* The ciliary projections into the lumen described in other species of Moniezia have not been observed.
2. *Non-cellular layer.* This layer possesses well-defined nuclei.
3. *Circular muscle layer.*
4. *Longitudinal muscle layer.*
5. *Parenchyma.* The cells of which are large, round or oval in shape, and possess well defined nuclei.

6. *Circular muscle layer.* With large, deeply staining nuclei.

7. *Longitudinal muscle layer.*

The wall of the vas deferens consists of a thin basal membrane, which is invariably surrounded externally by a well defined layer of prostatic cells with large nuclei. The layer is one or two cells thick, the constituent cells being very similar in appearance to those of the shell gland.

The testes are round or oval bodies varying in diameter from ·075 mm. to ·1 mm. They are tightly packed with small, darkly staining nuclei. Cavities in the testes, in which sperms may be observed, are frequent.

FEMALE ORGANS.

The vagina runs inwards from the genital papilla for ·3 mm. with a diameter of ·016 mm. It then dilates to form a short bulb, ·04 mm. long and ·02 mm. broad, which is separated by a constriction, ·02 mm. long and ·004 mm. in diameter, from the receptaculum seminis.

The latter is a large, thin-walled sac, ·5 mm. long and ·1 mm. in diameter, which runs inwards and slightly posteriorly, towards the shell gland. A short distance from the median extremity of the receptaculum seminis, and on its ventral side, a large duct, ·016 mm. in diameter, takes its origin. This duct immediately constricts to a diameter of ·008 mm., the constriction being followed by a dilated portion ·02 mm. in diameter. This duct is exceedingly muscular, its wall consisting of diagonal muscle fibres with well defined nuclei, surrounded by an outer layer of longitudinal muscle fibres. At the dilated portion referred to above, the duct divides into a ventral branch, the oviduct, and a dorsal branch leading to the shell gland. This point of bifurcation is ·016 mm. from the point of origin of the duct.

The oviduct is ·008 mm. in diameter. It consists of a basement membrane, and an internal epithelium, the constituent cells of which are large, and possess well defined nuclei. It follows a curved course, the convexity being ventral, and runs inwards anteriorly and ventrally, to enter the central portion of the ovary, from which it is separated by a constricted portion ·004 mm. in diameter, and a short bulb ·006 mm. in diameter. Both constriction and bulb have very muscular walls.

The shell gland duct is similar to the oviduct in structure, it is ·008 mm. in diameter. It runs dorsally, slightly inwards, and towards the posterior border of the segment, to enter the shell gland, in the centre of which it dilates to ·02 mm. From this dilation, two ducts take their origin, the vitelline duct, and the uterine duct.

The vitelline duct is ·004 mm. in diameter. It arises from the ventral portion of the dilation, runs a short distance ventrally, and then turns abruptly posteriorly to enter the yolk gland, where it dilates suddenly to a diameter of ·02 mm. It performs no convolutions.

The uterine duct arises dorsally from the median end of the dilation in the centre of the shell gland. It is much coiled, both inside and outside the latter. It runs dorsally for a short distance, then curves anteriorly and runs ventrally, to open into the uterus almost in the median line.

The vagina wall is similar in structure to the cirrus wall, the muscle layers, however, being in the reverse order. It consists of the following layers:

1. *Lumen.*
2. *Homogeneous layer*, possessing well defined nuclei.
3. *Longitudinal muscle.* Better developed at the median end than at the lateral end.
4. *Circular muscle.* Better developed at the lateral end than at the median end.
5. *Cellular layer* of large rounded cells with well defined nuclei.

The wall of the receptaculum seminis consists of a basement membrane enclosing a homogeneous layer with well defined nuclei.

The ovary is renal in form, its two extremities enclosing the shell gland and vitelline gland. It occupies a ventral position. Its average lateral dimension is 1 mm. and its diameter ·20 mm., both these dimensions varying considerably according to the degree of contraction of the segment. The cortical portion consists of a number of long, slender tubes, densely packed with large darkly staining nuclei, and radiating in a fan-like manner from the medullary portion, from which the oviduct takes its origin.

The shell gland is a compact organ, circular or oval in shape, lying in the median plane between the ovary and the vitelline gland. Its diameter varies from ·09 mm. to ·16 mm. It is composed of well defined or oval cells, tightly packed together and possessing large and well defined nuclei.

The yolk gland lies on the posterior side of the ovary and the shell gland, and slightly ventral to the latter. It is composed of a number of short, wide tubes, radiating from the central cavity from which the vitelline duct takes its origin. It is packed with darkly staining nuclei rather smaller than those of the ovary. The gland is oval in shape, its lateral diameter measures ·24 mm. and its transverse diameter varies from ·16 mm. to ·10 mm.

The uterus is a most complicated, much branched organ, the branches ramify throughout the entire segment, crossing the excretory canals dorsally to enter the lateral fields. The uterus wall is composed of a basement membrane.

OVA.

The eggs are more or less globular, tending to become cuboid on pressure. They vary in diameter from ·044 mm. to ·077 mm. The embryo, in common with that of the other species of the genus, is surrounded by a pyriform apparatus, the bulb of which immediately surrounding the embryo varies in diameter from ·022 mm. to ·029 mm. The bulb is prolonged into a pair of horns ·012 mm. in length, ending in a disc ·009 mm. in diameter.

SURVEY OF THE GENUS *MONIEZIA* IN RELATION TO THE NEW SPECIES.

The species of Moniezia are divided into the following sub-groups:

A. Interproglottidal glands absent—*Alba Group.*

B. Interproglottidal glands present, arranged in a linear manner, not around blind sacs—*Planissima Group.*

C. Interproglottidal glands present, arranged around blind sacs—*Expansa Group.*

The new species, therefore, since it possesses linear interproglottidal glands, belongs to the Planissima Group. The members of this group, together with those species of Moniezia in whose description no mention of interproglottidal glands has been made, are detailed below. In each case, the characters which distinguish them from the new species are given.

M. amphibia (Linstow 1901)(1).

Interproglottidal glands not mentioned. Testes not confined to the posterior portion of the segment.

M. benedeni (Moniez 1879)(2).

In this species the interproglottidal glands are "linear but extremely indistinct (absent?)." The scolex is large and obtuse, measuring ·96 mm. broad and ·48 mm. long, *i.e.* twice as broad as long.

M. bipappilosa (Leidy 1875)(3).

The description of this species is too scanty even to warrant its inclusion in the genus Moniezia. It may, therefore, be disregarded.

M. caprae (Rudolphi 1810)(2).

The description is too scanty to allow acceptance as a true species. It may therefore be disregarded.

M. conjugens Schauter 1917 (4).

In this species the interproglottidal glands are "first linear, and later arranged around three blind sacs."

M. crassicollis Schauter 1917 (4).

Interproglottidal glands linear, but indistinct. Scolex 1·26 mm. broad. Strobila 45 cm. long.

M. crucigera (Nitzsch and Giebel 1866) (2).

The type specimen having been lost and the internal anatomy not having been described, there is no justification for including this species in the genus. It may therefore be disregarded.

M. latifrons Schauter 1917 (4).

Scolex very large, measuring 1·9 mm. in breadth.

M. neumanni Moniez 1891 (2).

In this species the testes are not confined to the posterior portion of the segment.

M. nullicollis Moniez 1891 (2).

Strobila 40 cm. long. Largest segments 8 mm. wide and 1 mm. long. Scolex 1·3 mm. in diameter. Interproglottidal glands not described.

M. parva Schauter 1917 (4).

Strobila 25–30 cms. long. Ripe segments 2·75 mm. broad, 1·25 mm. long.

M. pellucida Blei 1921 (4).

The testes in this species are arranged in two triangles.

M. planissima Stiles and Hassall 1893 (2).

In this species the testes are not confined to the posterior portion of the segment.

M. rugosa (Diesing 1850) (5).

This species has not been sufficiently described to make it certain that it belongs to the genus Moniezia. If it is, however, a true Moniezia, the fact that the testes are not confined to the posterior portion of the segment, would differentiate it from the new species.

M. triangularis Marotel 1912 (6).

In this species the testes are arranged in two triangles.

M. vogti (Moniez 1879) (2).

This species is not sufficiently described even to warrant its inclusion in the genus Moniezia. It may therefore be disregarded.

The above forms a complete list of the species of Moniezia if the following are added:

Alba Group.

M. alba (Perroncito 1879).
M. denticulata (Rudolphi 1810).

Expansa Group.

M. expansa (Rudolphi 1810).
M. minima Marotel 1912.
M. oblongiceps Stiles and Hassall 1893.
M. trigonophora Stiles and Hassall 1893.

It is clear, therefore, that the species is correctly regarded as new, and hitherto undescribed. I propose for it the name *Moniezia translucida* n.sp.

SPECIFIC DIAGNOSIS OF *MONIEZIA TRANSLUCIDA* N.SP.

Adult strobila 150–200 cms. long, colourless and translucent in the anterior portion, yellowish in the mature portion. Scolex small, pear-shaped and always longer than broad; ·625–1·25 mm. long, ·496–·750 mm. broad, tapering gradually into neck. Suckers at four corners, running diagonally forwards. Neck long and slender, 2·38–4·63 mm. long, ·214–·496 mm. broad. Genital organs first visible 15–20 cms. from scolex. Segments always much broader than long, mature segments attain 12 mm. in breadth and 3 mm. in length. Interproglottidal glands linear and distinct. Genital pores double, situated in the anterior half of the lateral margin. On the right, vagina ventral and usually posterior, cirrus dorsal and usually anterior; on the left, vagina dorsal and usually anterior, cirrus ventral and usually posterior. Genital canals pass dorsal to longitudinal excretory canals and nerves. Testes arranged in a long narrow rectangle as numerous in the median line as elsewhere, but absent from the lateral fields, always confined to the posterior portion of the segment and always in a single dorsal layer. Uterine folds enter the lateral fields. Eggs ·044 mm.–·077 mm. in diameter. Bulb of pyriform apparatus ·022–·029 mm., horns ·012 mm., disc ·099 mm.

REFERENCES.

(1) VON LINSTOW (1901). *Jenaische Zeitschr. f. Naturwiss.* v, 35, n.f.v. 28 (4), pp. 409–428; Plates XIII and XIV, figs. 1–34.

(2) STILES, C. W. and HASSALL, A. (1893). U.S. Dep. Agric., Bureau of Animal Industry, *Bull.* No. 4.

(3) LEIDY, J. (1904). *Smithsonian Misc. Collec.* XLVI, No. 1477.

(4) BLEI, R. (1921). Centralblatt für Bakteriologie, Parasitenkunde und Infektionskrankheiten. Erste Abteilung. *Medizinisch-hygienische, Bakteriologie und tierische Parasitenkunde Originale,* Band 87, Heft 5. Gustav Fischer, Jena, December 17th.

(5) LÜHE, M. (1895). *Arch. f. Naturg.* 1895, pp. 199–212.

(6) MAROTEL, G. (1913). 9th *congr. intern. Zool. Monaco,* 1913, pp. 662–663.

EXPLANATION OF PLATE XV

Fig. 1. Scolex.

Fig. 2. T.S. mature proglottis, right half. 1. Cirrus. 2. Cirrus pouch. 3. Vagina. 4. Vas deferens. 5. Prostatic cells. 6. Sperms. 7. Ventral excretory canal. 8. Dorsal excretory canal. 9. Receptaculum seminis. 10. Portion of uterus containing eggs. 11. Cuticle. 12. Sub-cuticular layer. 13. Longitudinal muscle layer. 14. Circular muscle layer.

Fig. 3. Mature proglottis—pre-uterine stage. 1. Genital papilla. 2. Cirrus pouch. 3. Vas deferens. 4. Vagina. 5. Receptaculum seminis. 6. Ovary. 7. Vitelline gland. 8. Shell gland. 9. Interproglottidal gland. 10. Testes. 11. Transverse excretory canal. 12. Dorsal excretory canal. 13. Ventral excretory canal.

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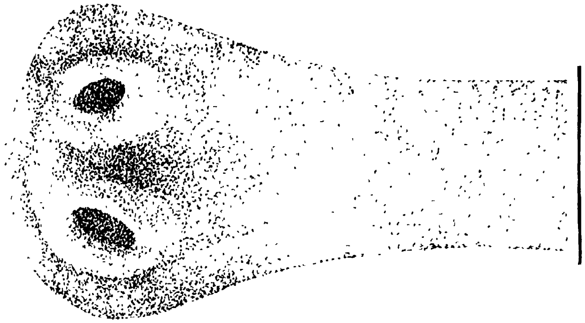

Fig. 1.

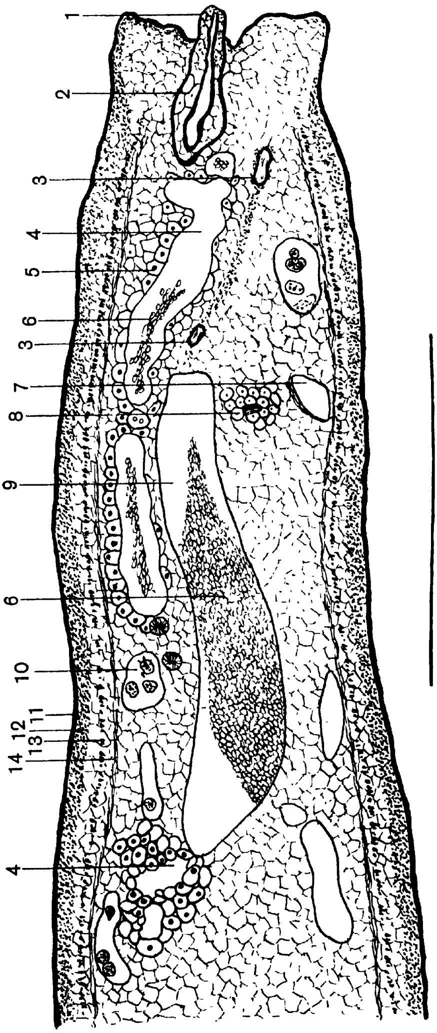

Fig. 2.

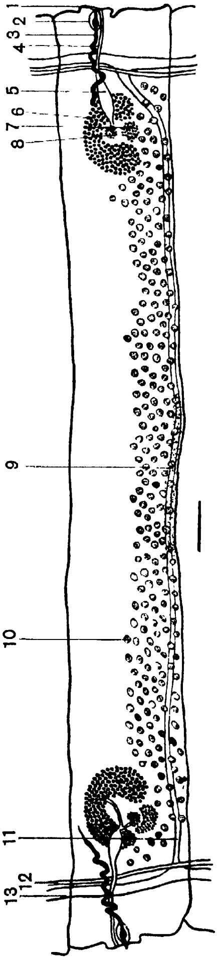

Fig. 3.

JENKINS.—On a New Species of Moniezia from the Sheep. (pp. 276—286.)

STUDIES ON THE SEX-RATIO AND RELATED PHENOMENA

(3) NOTE ON THE INFLUENCE OF SIZE OF LITTER

By A. S. PARKES, B.A. (Cantab.).

(Zoological Department, University of Manchester.)

(With 1 Text-figure.)

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INTRODUCTORY.

The new data which are recorded in this paper have been compiled from the *Duroc Jersey Pig Herd Book*, Vol. 67. In this volume the number in the litter and the sexes at weaning time (presumably about seven weeks old) are given. Thus, if any of a litter die before weaning time the litter is not completely accounted for in the numbers of the sexes. This happens in a large percentage of cases, but for purposes of this investigation only those litters were included where the original sex constitution could be obtained.

I am much indebted to Miss M. Parkes for assistance in the analysis of the records.

OBJECTS.

It is hoped later to be able to complete a more detailed and analytical study on the subject; but in view of the time required for such work this preliminary note is put forward as a statement of what appears to the writer to be the nature of the problem of the possible influence of litter size on the sex-ratio, and as an attempt to correlate the existing work on the subject.

PREVIOUS INVESTIGATIONS.

Most of what little previous work there is has been done on animals which are normally polytocus. Some information exists, however, relating to the sex-ratio of multiple births in normally monotocus animals.

Pearl (7), for instance, notes a decline in the sex-ratio of twins and triplets in man.

That of twins was slightly below normal, while that of triplets was remarkably low at 54·8 males per 100 females.

For triplets in sheep Pearl found 215 females to 130 males, a sex-ratio of 60·6[1].

From these and similar figures it has been suggested that multiple births tend to produce an excess of females.

Newcomb (5), however, found nothing abnormal in the sex-ratio of dizygotic twins in man.

Wentworth (9), too, found that 146 cases of triplets in sheep gave 226 males and 212 females, a sex-ratio of 106·5, which is very near what is probably the normal ratio for sheep. Wentworth, also, working on pigs and dogs, found that there was nothing very unusual in the sex-ratio of multiple births.

This conclusion has also received support from King's (3) work on rats.

Parker and Bullard (6), working on rather different lines, give the following table showing that the size of the litter in pigs has little effect on the sex-ratio:

Table I.

Litter size and sex-ratio, from Parker and Bullard.

Number in litter	Males	Females	Sex-ratio
1	9	6	150
2	54	46	117·5
3	110	112	98·2
4	272	264	117
5	331	354	93·7
6	578	556	104
7	610	538	113·4
8	479	449	106·7
9	269	280	96·1
10	148	172	86
11	78	87	88·8
12	31	41	75·6
13	24	15	116
14	23	19	112·1
15	8	7	110·4
Totals	3024	2946	102·5

---

[1] All sex-ratios are calculated as males per 100 females.

These results show that the influence, if any, of the size of litter on the sex-ratio is small and is in any case erratic. Parker and Bullard came to negative conclusions from the results.

From Machens' excellent paper (4) the following data are taken for litter sizes at which ten or more litters were found:

Table II.

Litter size and sex-ratio, from Machens.

Size of litter	5	6	7	8	9	10	11	12	13	14
Sex-ratio	100	92·8	96·4	100	96·9	102·6	96·5	103·5	111·5	127·6

These again show an unfortunate incoherence.

It may be noted, however, that the sex-ratio of the total, 102·7, is remarkably similar to the result obtained by Parker and Bullard.

PRESENT DATA.

My own material tabulates out as under:

Table III.

Litter size and sex-ratio in Duroc Jerseys.

Size of litters	No. of litters	Males	Females	Total	Sex-ratio	% of total litters
4	26	59	45	104	131	2·7
5	54	155	115	270	134·8	5·5
6	110	338	322	660	105	11·4
7	186	689	613	1302	112	18·3
8	203	856	768	1624	111·5	20·9
9	175	783	792	1575	99	17·8
10	138	692	688	1380	100·6	14·2
11	63	355	338	693	105	6·4
12	27	180	144	324	124·5	2·8
Totals	982	4107	3825	7932	107·3	100

Litters of more than 12 and less than 4 are left out owing to being too few to give a reliable result.

The number of litters and the sex-ratio at each litter size are conveniently shown on a graph (Fig. 1).

DISCUSSION.

In all the cases mentioned above the importance of the results depends on the nature of the problem which the investigation was meant to elucidate. The early work on twinning was designed to throw light on the supposition that sex was determined by influence during gestation.

If this were so, twins, being developed under similar conditions, would be expected to show a greater proportion of unisexual births than would be expected by chance.

This old theory of sex-determination is hardly tenable now, and the later work on the sex-ratio and litter size seems to have been without any definite object.

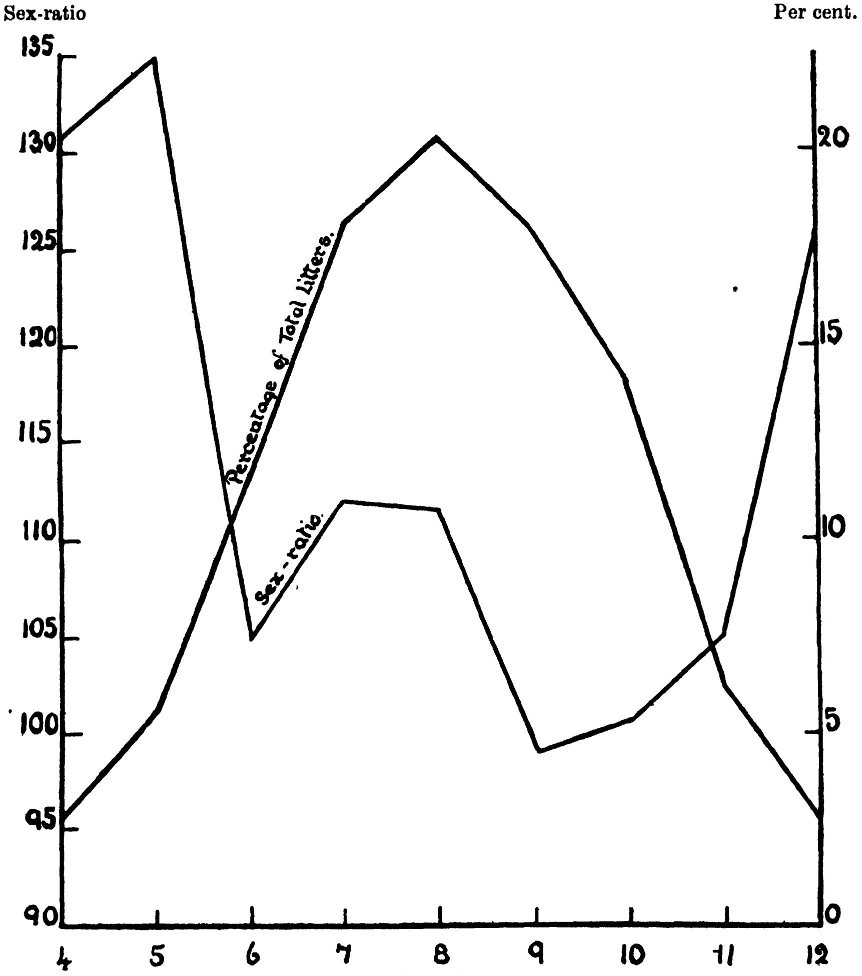

Fig. 1. Sex-ratio and percentage of total litters of each litter size.

The results mentioned above are far too indefinite to discuss theories of sex-determination upon, but viewed from other angles they are extremely interesting. From Wodsedalek's (10) work it seems eminently probable that the modern tendency to locate sex-determination at fertilisation applies equally to the pig and other mammals, and this may be taken as a tentative basis for analysis of the above results.

Hammond (1) has shown that the number of full time foetuses in the pig is far less than the number of conceptions. This author found 49 atrophic foetuses to 267 normal ones, which is a mortality *in utero* of 15·5 %. Hence the size of a litter at birth is governed by two factors:

(1) The number of eggs fertilised.

(2) The mortality between conception and birth.

Now, on the current theory of sex-determination it is very hard to see how the number of eggs fertilised can affect the sex-ratio; but there is abundant evidence that the amount of foetal elimination between fertilisation and birth can affect the sex-ratio at birth. Schultz (8) for man, Jewell (2) for cattle, and my own observations on mice[1], have shown that in these three animals at least antenatal mortality falls preponderatingly upon the males.

We have now practically reduced the problem to whether or not the size of the litter can affect the amount of foetal elimination. In considering this it is essential to separate multiple births in animals which normally only produce one at a birth from multiple births in animals where they are the rule. Genital organs designed for one foetus will clearly have a greater difficulty in accommodating themselves to a number of foetuses than a normal uterus will in accommodating a litter larger than the average.

In man, male foetuses are larger than female ones, and thus male triplets would have less chance of arriving to full time than female triplets would have, and this fact probably goes a long way to accounting for the remarkable sex-ratio given for triplets by Pearl.

The case of the normal multiple pregnancies is very different. If the size of a litter can be the product of one or both of two factors, one of which affects the sex-ratio and one of which does not, it is not difficult to see that the results must be erratic, and in the case of the Duroc Jersey records the coherence found is more than might be expected.

The future line of work lies in investigating the relative importance of the two factors which govern the size of the litter, and their interrelation to the sex-ratio.

[1] As yet unpublished.

SUMMARY.

(1) The existing work on the influence of the litter size on the sex-ratio shows very erratic results, and the new work embodied in this paper is rather more coherent.

(2) It is clear, however, that multiple births in animals which are normally polytocus are somewhat different from multiple births in animals which are normally monotocus.

(3) Before any attempt can be made to arrive at the nature of the influence of the size of litter on the sex-ratio it is essential to consider the factors which govern the size of the litter. Hammond (1) has shown that the number of eggs fertilised and the amount of foetal elimination are the two main factors controlling the size of litter. The first of these factors can scarcely affect the sex-ratio, while the second, on the other hand, very well may have such an influence.

(4) In view of the complexity of the interaction of these two factors any great coherence in the resulting sex-ratio could hardly be expected.

LITERATURE.

(1) Hammond, J. (1921). *Journ. Agric. Sci.* xi.
(2) Jewell, F. M. (1921). *Biol. Bull. of Marine Biol. Lab. Woods' Hole,* xli.
(3) King, H. D., and Stotenburg, J. M. (1915). *Anat. Rec.* xi.
(4) Machens, A. (1915). *Berliner Tierärztliche Wochenschrift.* xxxi.
(5) Newcomb, S. (1904). Carnegie Instit. Wash. Pub. No. 11.
(6) Parker, G. H., and Bullard, C. (1913). *Proc. Am. Acad. Arts and Sci.* 48.
(7) Pearl, R. Quoted by Wentworth.
(8) Schultz, A. H. (1918). *Biol. Bull.* xxxiv.
(9) Wentworth, E. N. (1914). *Sci.* N.S. 39.
(10) Wodsedalek, J. E. (1913). *Biol. Bull.* xxv.

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REVIEW

Practical Zoology for Medical and Junior Students. By Prof. J. D. F. GILCHRIST and C. VON BONDE. E. and S. Livingstone, Edinburgh, 1922. Price: 15*s*. net.

Prof. Gilchrist's book tends at first glance to create a very favourable impression upon the reader. The publishers have certainly carried out their part of the work very well; the paper and the printing are attractive; the binding would have rejoiced the heart even of Chaucer's clerke of Oxenforde; the hundred and five illustrations, if lacking the originality and beauty of those of Kukenthal or Jammes, are at any rate clear, and do possess the merit of having their component parts *named* and not numbered nor lettered; the book is interleaved with blank paper, intended, although glazed, apparently for sketches.

Deeper acquaintance, however, induces a feeling of disappointment. In a book which is intended to compete against such a well established favourite as Marshall and Hurst's text-book, we have a right to expect outstanding originality in choice and treatment of types. On the contrary, we find only two types which are not dealt with by Marshall and Hurst, and these two, the Sponge and the Sea Anemone, are thoroughly described in Parker and Haswell. In any case, the inclusion of the latter type in a book intended for First Year Students is of doubtful value when it is made at the expense of the more typical and the metagenetic type of Coelenterate, such as *Obelia*. The value of the Earthworm as a type is similarly reduced by the failure to precede its description by an account of a more typical Chaetopod, *Nereis* for example. There is not a line of Embryology, not a line of Histology, and in a book catering primarily for South African Students the omission of such types as a Haemoflagellate, a Muscid Dipteron, a Grasshopper, and a Lizard, is inexplicable.

The dissection directions are feeble in the extreme. A book on Practical Zoology, written in 1922, should describe methods which were unknown possibly to compilers of previous manuals. Fifteen shillings is a high price to pay for information that could be better obtained verbally from one's grandfather. There are, for example, at least two methods of exposing the Afferent Branchial System of the Elasmobranch which replace the notoriously inadequate method of Marshall and Hurst; there is a method of exposing the Sympathetic Nervous system of the Frog by dissection from the dorsal side; there is the tap-stream method of obtaining the alimentary canal and nerve cord of the Cockroach intact. Are these and many other points unknown to the compilers of this practical handbook, or are they unworthy of consideration?

On the whole, we cannot congratulate Prof. Gilchrist and his collaborator upon the book. A more judicious selection of types and a more careful attention to details of technique would have rendered such a work attractive and valuable to students of all grades, but as it is, in spite of many admirable features, it runs a risk of becoming regarded merely as an emasculated version of Marshall and Hurst.

R. A. W.

VOLUME X DECEMBER, 1923 NOS. 3 & 4

ON FIBRES OF "INTERMEDIATE" CHARACTER FOUND IN THE FLEECE OF *OVIS VIGNEI*

BY F. A. E. CREW AND J. S. S. BLYTH.

(*From the Animal Breeding Research Dept., University of Edinburgh.*)

(With Plates XVI and XVII and 3 Text-figures.)

IN an endeavour to define standards to which the fibres of the fleece of the domesticated sheep might be compared, one of us a year ago gave a brief description of a sample of the fleece of *Ovis ammon poli.* In this two sorts of fibre were present, *e.g.* "hair" and "wool," constantly and markedly distinct one from the other in their characterisation: no fibres showing any intermediate characterisation were found. But since that time we have examined a great number of samples of the fleeces of different modern domesticated breeds and consequently it was decided to make a further examination of the fleece of the wild species in order to find out whether in their fleece, as is commonly the case in the domesticated sheep, there is an "intermediate" fibre present.

To Professor Cossar Ewart, who was good enough to supply us with abundant material, we wish to express our thanks.

It was found that two samples, from *Ovis vignei* and *Ovis musimon*, differed from the rest in that instead of being composed entirely of two distinct types of fibre—"hair" and "wool"—they contained a few fibres the characters of which appeared to be intermediate between these two main groups. A detailed examination of the sample from *Ovis vignei* has been made and the description is as follows:

Ovis vignei (Plate XVI, fig. 1).

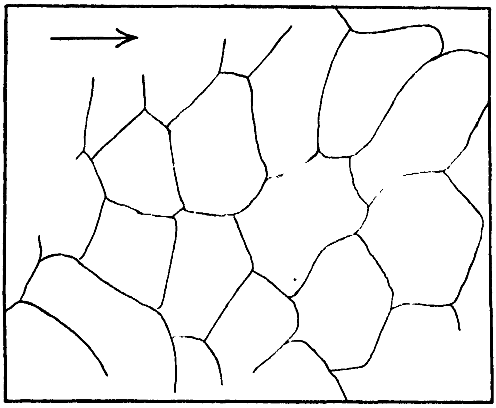
To the naked eye the sample appeared to be composed of the same two types of fibre found in the fleece of *Ovis ammon poli* and described previously: "hair"—thick, straight, stiffly waved, dead white in its proximal portion and creamy brown in its distal third; and "wool"—fine, springy, curly and of a pearly colour with occasionally a brownish distal half. The wool varied in length considerably, the hair but slightly. Careful examination revealed the presence of fibres of intermediate character. These fibres could be divided into two classes: (*a*) those which looked like hair but were shorter, less stiff, distinctly finer, and always pointed; and (*b*) those which were indistinguishable from wool in their fine, curly, proximal part, but showed a stiff, straight distal portion, white or pale brown in colour.

MICROSCOPICAL EXAMINATION.

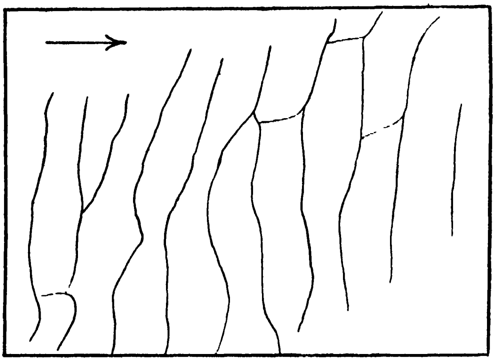
Wool (Plate XVII, fig. 7). These fibres were exceedingly fine in diameter, usually non-medullated in the proximal part, the shaft being composed, apparently, of cortex merging very near the surface into cuticle; the presence of the latter was indicated solely by the free edges of its external scales each of which encircled the entire circumference of the fibre. Tips were present in all fibres. Towards the tip the fibre broadened slightly and in a few short rod-like areas of air-filled medulla with no definite structure were seen. One or two fibres were pigmented throughout their entire length and a few others were lightly pigmented in the distal portion.

Hair (Plate XVII, figs. 4 and 6). The difference in diameter between the hair and the wool was very marked, that of the former being roughly fifteen times that of the latter. Unlike the wool, the hair had a diameter practically constant throughout the entire length of the fibre save at either end where it diminished slightly. The shaft of the hair consisted of a reticulate air-filled medulla surrounded by an exceedingly thin homogeneous layer identified as cuticle and cortex (*vide infra*) which, in the proximal portion of the fibre, showed slight notches on its outer surface. In the distal portion the place of the notches was taken by projecting scale-like points. In contradistinction to the wool and the "intermediate" fibre none of the hairs had intact tips but a few were practically complete proximally. In these it was found that the fibre narrowed and the medulla gradually diminished and finally disappeared, while the homogeneous layer increased to form the entire diameter of the fibre near the root. All the fibres of this kind were more or less free from pigment in their proximal portion, but distally pigment granules were present in increasing number towards the tip.

In the proximal part of the fibre the surface markings (Plate XVI, fig. 2) were reticulate, resembling very much those of the medulla (Plate XVI, fig. 3) and differing from them only in that while the medullary markings were thicker and coarser and the lines fused forming rough diamonds or irregular triangles, the more superficial markings were extremely delicate, being, in fact, invisible when anything but a $\frac{1}{12}$th oil-immersion was used, and ran together to form well defined clean-cut angles. Distally this type of surface marking merged into another which took the form of lines roughly parallel to one another, which ran unevenly across the fibre at right angles to its length and, when compared with typical wool surface markings, lay relatively closer to one another. In this part of the fibre the surface consisted of overlapping scales and their ectal edges projected on the lateral margin. Unlike the wool in which but one scale encircled the entire circumference there were here two to four scales. In the fibres which still retained the root it was seen that in this region distinct wool surface markings were present stretching from side to side and ending laterally in free projecting scales.



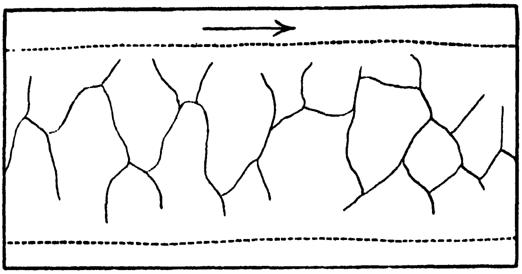
Proximal portion of fibre.



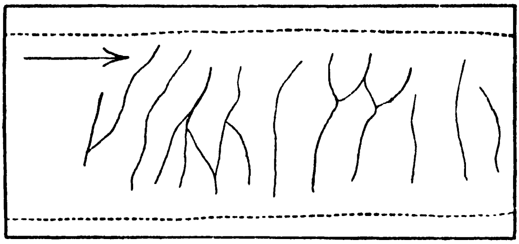
Distal portion of fibre.

Fig. 1. Surface markings on hair of *O. vignei Indicus* (arrow pointing in the direction of the tip of the fibre). × 350.

Intermediate fibres. (*a*) Those which on superficial examination resembled hair had much in common with it when examined microscopically. Fairly uniform in diameter, the medulla of this type was still very large while the homogeneous area surrounding it was relatively thicker. Distally where the fibre began to taper the medulla diminished in size and finally disappeared leaving the homogeneous layer to form a fine tip resembling that of wool. On the surface the lateral edges showed free projecting scale tips throughout except towards the proximal end of some fibres where only notches were to be seen. The surface markings

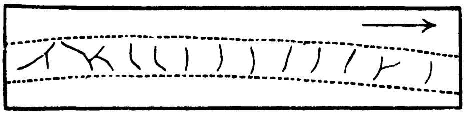


Proximal portion of fibre.

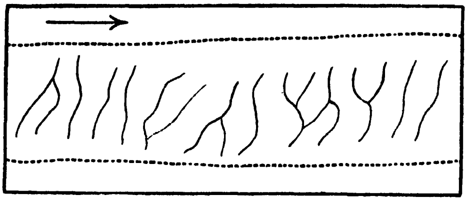


Distal portion of fibre.

Fig. 2. Surface markings on Intermediate (*a*) fibre of *O. vignei Indicus* (arrow pointing in direction of tip). ×350.



Proximal portion of fibre.



Distal portion of fibre.

Fig. 3. Surface markings on Intermediate (*b*) fibre of *O. vignei Indicus* (arrow pointing in direction of tip). ×350.

were like those of the hair. A few of the fibres possessed a fine non-medullated root portion.

(*b*) In those fibres which resembled wool rather than hair, the proximal portion was very similar to wool microscopically. The medulla, however, appeared as rod-like pieces which in the distal portion of the fibre became continuous and widening, as the fibre itself increased in diameter to assume the appearance of the finer fibres of the previous intermediate type (*a*), becoming reticulate and forming a great part of the shaft of the fibre with only a narrow homogeneous superficial layer. The surface markings throughout appeared to be similar to those of typical wool.

In the wool and finer intermediate fibres it was possible to identify cuticle, cortex and medulla, although it was impossible to draw a sharp line of demarcation between these parts. In the case of the hair and the thicker intermediate fibres, however, examination after the ordinary methods of treatment failed to convince us of the presence of cortex. It was found, however, that if, after washing, the fibre was mounted in sulphuric acid and gently heated it was possible by pushing the cover-glass gently along the slide to slough off layers from the surface of the fibre; in some fibres so treated the cuticle came off in large extremely transparent sheets, showing clearly the surface markings. Between this and the main shaft another clear layer appeared showing no transverse markings but having very faint longitudinal striations along which, on further movement of the cover-glass, it tended to break up. The rest of the fibre showed medullary markings. The central layer which was taken to be cortex was more definitely seen in fibres from species with heavily pigmented fleece (Plate XVII, fig. 8) since in these the pigment granules would appear to outline the cortical cells. The homogeneous part at both ends of the fibres was found to fray out into long thin rods similar to this central layer and may therefore be concluded to be also cortex. Ordinary commercial sulphuric acid was found more satisfactory for our purpose than a dilute solution, as the latter seemed to cause more thorough disintegration, cells coming off singly instead of in layers.

The surface markings of the hair fibres are not corticular, as was suggested in the description of the fleece of *Ovis ammon poli*: they are either medullary or cuticular.

As to the significance of the intermediate fibre: it cannot be regarded as an undeveloped hair, since it has been shown that hair growing, as it does, during summer is more or less uniform in length in the winter-coat. The fact that a few of these fibres were complete and therefore ready to shed also supports the evidence against this contention. There is no suggestion that the intermediate fibre is the common type from which both hair and wool later develop: the two types of intermediate fibres found in the fleece of *Ovis vignei* are constantly distinct. One (type *a*) is always uniform in diameter, the other (type *b*) always thicker in the distal than in the proximal portion; there is no evidence of any transformation of the one type into the other. On the strength of that it would seem improbable that the intermediate type is a modification of only one type of fibre—only hair or only wool—though it would seem possible that it is a modification of both.

Table I. *O. vignei.* Variation in the Diameter of Fibres* (Centimetres).

	Intermediate fibres		
Hair	*a*	*b*	Wool
0·0919	0·0323	0·008281	0·005733
0·0864	0·0266	0·009018	0·005733
0·0946	0·0583	0·009018	0·005642
0·0915	0·0505	0·009018	0·005460
0·0925	0·0226	0·009100	0·005460
0·0939	Mean =	Mean =	0·005278
0·0965	0·0381 ± 0 0042	0·008887 ± 0·00008	0·005187
0·0901			0·005187
0·0825			0·005005
0·0873			0·005005
0·0826			0·004914
0·0958			0·004823
0·0941			0·004277
0·1006			0·004095
0·0987			0·003913
Mean =			Mean =
0·0916 ± 0·0009			0·005047 ± 0·000095

* Measurements of diameters were taken in the proximal half-inch of the fibre.

Table II. *O. vignei.* Variation in Length of Fibres.

		Intermediate type		
	Hair	*a*	*b*	Wool
No. of fibres in sample	50	14	15	236
Shortest fibre... ...	3·3	2·3	2·05	0·65
Longest fibre... ...	3·8	3·5	2·8	3·55

DESCRIPTION OF PLATES XVI AND XVII

Fig. 1. Sample of fleece of *Ovis vignei Indicus.* × 2.
Fig. 2. Cuticular markings on hair. × 700.
Fig. 3. Medullary markings on hair (same field). × 700.
Fig. 4. Longitudinal section of hair. × 105. Left section near lower surface showing cortex below and medulla above.
Fig. 5. Longitudinal section of intermediate fibre. × 105.
Fig. 6. Cross-sections: above—thick intermediate fibre; below—hair. × 105.
Fig. 7. Wool of *Ovis vignei,* optical section. × 105.
Fig. 8. Portion of hair from *O. musimon,* treated with sulphuric acid. × 200.

ct = cuticle; *cr* = cortex; *m* = main shaft of fibre showing medulla.

(*Received May* 21*st*, 1923.)

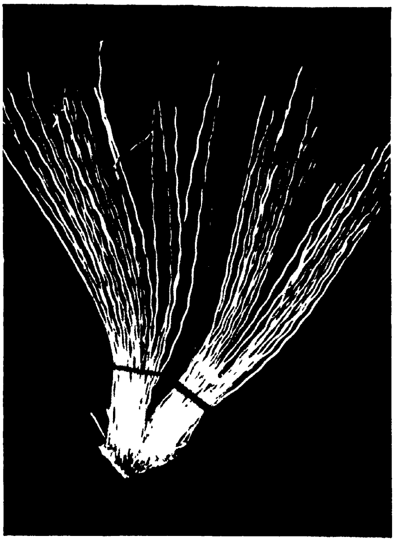


Fig. 1.

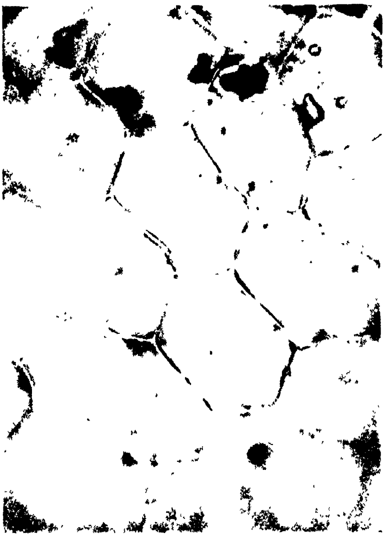


Fig. 2.

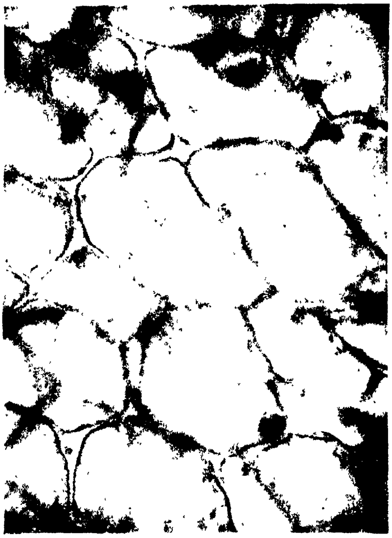


Fig. 3.

CREW & BLYTH.—On Fibres of "Intermediate" Character found in the Fleece of *Ovis vignei*. (pp. 295—300.)

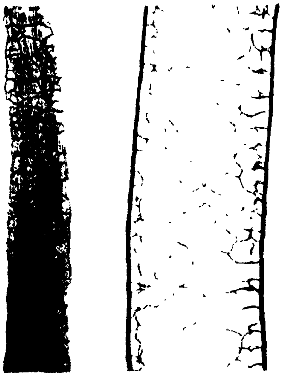


Fig. 4.

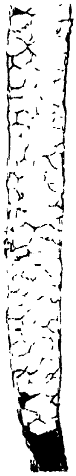


Fig. 5.

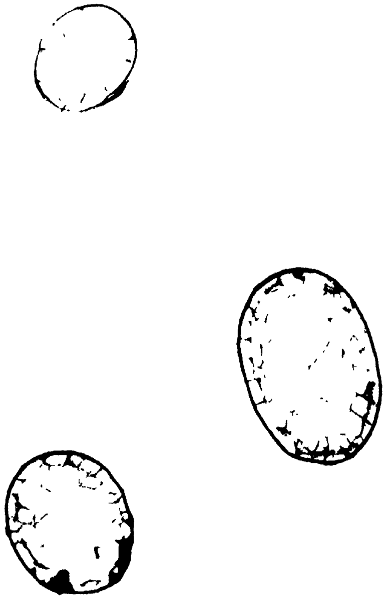


Fig. 6.

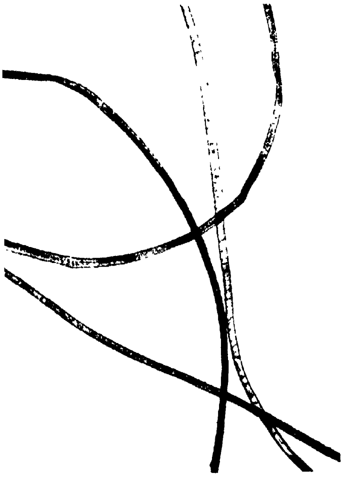


Fig. 7.

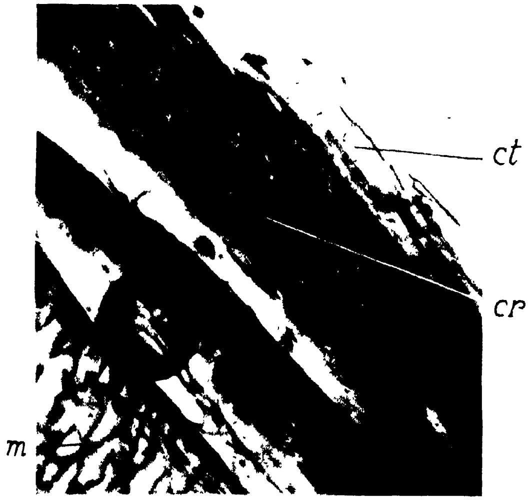


Fig. 8.

CREW & BLYTH.—On Fibres of "Intermediate" Character found in the Fleece of *Ovis vignei*. (pp. 295–300.)

MICROLOGICAL ANALYSIS OF TWO FLEECES FROM BLACKFACE SHEEP

By JANET S. S. BLYTH, B.Sc.

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(With Plate XVIII and 4 Text-figures.)

Partly on account of the wide distribution of the Blackface Mountain Sheep and partly owing to the practice of confining to each hill-grazing its own indigenous stock, there is, as might be expected, a wide variation in the type of fleeces from the various flocks of this breed. It being, therefore, quite impossible to take any particular fleece as typical of the breed, a skin and a fleece were used for the purpose of this paper. The skin (1) was from a show ram which had died in September, and, having missed the summer clip, the fleece was longer than usual. In appearance it was of the strong "hairy" quality commonly seen in the showyard sheep except for a small patch on the right shoulder where the long hair fibres had almost entirely given way to the much finer fibres (wool). In this patch the undercoat was visibly coarser than when completely covered by the long outer coat as was the case throughout the rest of the fleece. Kemp was found all over the fleece especially in the hind quarters.

The fleece (2) was the ordinary commercial type cut—it is understood—at the usual time. It was somewhat "open"; the hair fibres were much finer than in the previous case and did not by any means cover up the woolly undercoat although longer and greater in calibre. Only a very little kemp was present in the forequarters but it was very abundant in the hindquarters.

Samples taken from the shoulder and britch showed that the fibres could be classed into three types:

Easily detected by the contrast which its dead-white colour makes against the creamy brown colour of the rest of the fleece the first type, kemp, is found in considerable quantity throughout fleece (1). It is very brittle, many fibres having a number of "breaks" or bruised parts along their length and they usually have a peculiar stiff waviness. Shorter than the other constituents of the fleece kemp varies from ·75 inch to six inches, with most of the fibres about four inches long; all end in a fine tip except those which, very much bruised and broken, lie at different angles to the general direction of the fleece. Most of the longer fibres are loose and unattached to the skin, lying in the "matt" formed by the other two types, with their proximal ends, which taper down finely to terminate in a slight bulb, lying slightly nearer the skin than the fine portion in the next type (*v. infra*). The remaining fibres still attached to the skin do not possess a fine proximal portion except for one or two of an obviously pathological nature, which are brown, shrunk, twisted, and flattened for most of their length.

The second and longest type of fibre, hair, in fleece (1) were coarse and straight except where most had a single loop or waved part about three inches from the proximal end. They overlapped by several inches the other two types. Nearly all were more or less uniform in length, only a few having tips and these were either slightly shorter than the average (which terminate in a brown, much worn part) or else about three to four inches long when they were difficult to distinguish from kemp except by the latter's greater calibre and stiff waviness. About three inches from the proximal end (*i.e.* in the region where the looping occurs) a slight thinning could be discerned on the majority of fibres. Occasional loose fibres were found and were generally complete proximally with the root as in the case of kemp lying in the region of the loop on the other fibres.

In this fleece there is a distinct gap between this type and the next which comprises the finer shorter fibres of the woolly undercoat. For the most part these are curly with straighter, thicker distal parts either ending in a loosely waved portion or having the most distal half inch of their length straight. Tips are only found in one or two cases. A very few extra long fibres resemble the average distal portion throughout their whole length; on the other hand some fibres shorter than the rest are seen to be curly throughout, and among these only a few tips are present. Loose fibres occur; these are generally complete and as in the case of the other loose fibres they are shorter proximally ending in the region of the thin part of the hair.

Pigmented kemp fibres were found scattered throughout the whole of the fleece but coloured fibres of the other two types only occurred in small patches on the edge of the fleece.

In fleece (2) the three types of fibre present in fleece (1) are also found but with the following differences.

Kemp is only occasionally found as a complete fibre—that is the tapering proximal portion is generally absent. Those which are complete, however, terminate close to the cut edge of the fleece.

The long hair fibres are finer than in fleece (1) and while they are on the average distinctly longer than the wool the difference between the groups is less marked, one or two fibres being found which cannot be definitely allocated to either group. There is no loop on the fibres and individual fibres seem fairly uniform in bore throughout their length except at the proximal end where they are as a rule finer. Many of the fibres are straight and only slightly curved; others slightly finer have their proximal half very loosely curled.

Wool fibres appear to be much as described in fleece (1) with the exception that tips which are only occasionally present are found only among the longer fibres. The few complete fibres of both hair and wool which are present are found to terminate proximally near the cut edge of the fleece.

Pigmented fibres formed here and there throughout this fleece were found to be of all three types.

MICROSCOPICAL EXAMINATION.

For the purpose of microscopical examination numerous fibres were treated in a number of different ways according to the results desired. In all cases the fibres were first washed in methylated ether to remove the grease and foreign matter with which they are coated. By staining in picroformol and mounting in balsam a general view of the fibre in optical section was obtained. A series of fibres so treated were used for the preparation of the table of diameters (Table III). Longitudinal and cross-sections were obtained, after staining with picroformol, by embedding in paraffin wax and keeping the blocks in water for, at least, a week before cutting. To bring out the surface markings more clearly Hausman's method of dipping the fibre in an alcoholic stain was adopted. Solutions of various stains were used differing in strength both as regards alcohol and stain, but in all cases the results were irregular. Gentian violet and eosin appeared to give the best results. To ascertain in doubtful cases whether cortex and cuticle were present fibres were treated in sulphuric acid.

Kemp (Plate XVIII, fig. 1). The proximal portion is found to consist of an apparently homogeneous cortex, non-medullated, comparatively fine, which is fairly uniform in diameter throughout its (short) length. Here and there small black marks are seen dotted over it and towards the end these are very numerous, giving the appearance of pepper shaken from a pot. On careful focussing, they are seen to be tiny air-filled holes which lie not only on the surface but apparently all through the shaft. The end itself is frayed into hundreds of tiny narrow rod-like pieces and projecting ectally with their free ends pointing towards the skin.

Travelling distally the homogeneous portion broadens, a black (air-filled) rod-like medulla, occasionally discontinuous at first, appears and growing in breadth much more rapidly than the fibre itself soon fills almost the entire shaft, the homogeneous portion being represented in longitudinal section by a very narrow band running up each lateral edge. Soon after the medulla begins to broaden, its structure is indicated by the manner in which the air appears to lie in a collection of partly fused bubbles and where in some fibres the air has been expelled by the balsam the reticulate spaces typical of a broad medulla are easily seen sttetching from side to side of the medulla and showing several layers deep with occasional septae hanging between. Cross-sections also show the medullary network broken up by torn septae. The width of cortex in individual sections is always fairly uniform.

This picture now holds good for the main length of the fibre with exceptions that "breaks" occur at intervals. At these the regular pattern of the medulla becomes confused and usually the lateral edges of the hair jut out in two distinct points. The fibre remains fairly uniform in bore until more than half its length has been passed over; after that it decreases very slowly in size till about half an inch from the distal end it rapidly tapers out to a fine tip, the medulla thinning out more rapidly than the rest of the fibre, so that it ends half way to the end while the cortex increases in size, filling the whole shaft and then thins slowly to form the long fine tip.

On the lateral edges of the fibre a little way from the frayed proximal end free protruding edges of scales are seen in longitudinal section. Travelling distally these are followed at small intervals by similar scales until the fibre has attained its full width where they appear to be gradually replaced by vaguely raised ectal portions or notches. This holds until in the distal half notches are again replaced by projecting scales which are seen right out to the extreme tip of the hair.

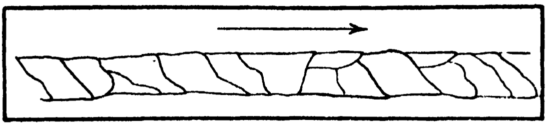
An examination after treatment for surface markings (Fig. 4) showed that these were of various types and corresponded but roughly with the different portions of the fibre. The most proximal markings (*i.e.* those over the homogeneous part of the shaft) run irregularly across the fibre from one side to the other, roughly parallel but occasionally bending to run into one another, forming a series of single overlapping scales as in typical wool. These gradually, as the fibre attains its full width, merge into another distinct type of markings which are of reticulate appearance; the meshes being four or five sided and roughly square, they generally lie diagonally to the length of the hair (*i.e.* with a corner pointing distally). Further on these broadening gradually merge into another type usually found in the distal half where the fibre is slightly thinner. These are very broad shallow scales and one or two to the breadth of the fibre may be seen. Finally, these merge into the markings of the tip where each scale more or less surrounds the circumference of the fibre. The edge of these scales shows up as an exceedingly irregular and jagged line running unevenly across the fibre.

Hair (Plate XVIII, figs. 2 and 3). Individual fibres of this type showed much more variation in calibre than in the last. The air-filled medulla, reticulate throughout and still large in the majority of cases, is smaller and the cortex broader than in the case of kemp. In the finer fibres, as also in the thin portions of the others, the medulla usually thins down considerably and may appear to lose its structure showing as a solid rod—continuous or discontinuous, while the cortex may broaden slightly at the expense of the medulla. The cortex is of homogeneous appearance in this type except in a few cases where, at the distal ends, it has been split up through weathering into rod-like pieces much as in the proximal end of the complete kemp fibre, but here we have a full-size medulla present right to the end and fraying is not so irregular. The cortex in longitudinal section is represented by two narrow bands, one lying up either side of the medulla. These are often of unequal width and this is also borne out in cross-sections where it is seen that, as a rule, the cortex is thicker on one side of the fibre than on the other. While some of the complete fibres ended proximally in a short non-medullate portion running out to end in a bulb of cortex frayed up into separate cells, others had the portion covered by a shrunken twisted sac, apparently the hair follicle.

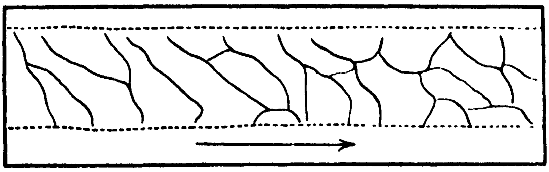
Surface Markings (Fig. 3). On the thickest part of the fibres the markings tend to be like those of kemp at its widest part; generally, however, their length lies along the direction of the hair or at a slight angle to it rather than across the breadth. As the hair narrows this lengthening is more obvious, but the appearance of overlapping scales which can be seen in the first case is less obvious here and, in fact, the contour of the scales would often lead one to believe that they overlapped in the opposite direction, did not those on the lateral margin of the hair with their ectal edges pointing distally belie this. Very fine portions of fibres of this type such as shown by some fibres at the "nick" (or thin portion) show a further lengthening of the scales; these lie diagonally across the fibre in the main tending slightly to lie parallel with its breadth. Although in some cases lying at right angles to the length these markings are never at any time like those in the distal half of the kemp fibre, since the impression of regularity obtained from the latter is never given here.

Where tips were present these were formed as in the case of kemp, the homogeneous cortex running out to the tip surrounded by cuticular scales, each sitting in the cup of the other as in typical wool. It was noted that in many of these fibres the distal end, when treated for surface markings, presents a false picture in that, being very translucent and having lost most of its cuticle by attrition and other weathering processes to which it has been subjected, the medulla shows up as if it were on the surface and may be mistaken for cuticular markings. Especially is this the case when eosin has been used, as it is readily picked up by the medulla.

Wool (Plate XVIII, figs. 4, 5 and 6). This varies in calibre both within its type and within individual fibres. The fibres in fleece (1) are thick for about an inch of their length proximally, then thin down and, remaining fine for a considerable part of their length, they thicken again till the straighter distal portion is reached; this latter part is fairly uniform in diameter. In fleece (2) no thick proximal part is found. The thickest parts of this type of fibre are as thick and in occasional cases thicker than the finest portions of "hair"; also they approach these in structure, possessing an air-filled rod-like medulla and a comparatively thick cortex covered by projecting cuticular scales. In optical section the medulla shows no structure but longitudinal and cross-sections show, although somewhat vaguely, that an arrangement of cells is present. Apparently these are placed regularly one above the other and in diameter the medulla may be one or two cells deep. Throughout the thick portion it is in the main continuous, being discontinuous during the transition from the thick to the thin portion of the fibre. Here depending on the average size of the fibre it may either disappear altogether or fragments of medulla may appear irregularly down the centre of the fibre. Surface markings (Fig. 1) in the finer part, especially if non-medullated, are those of typical wool, *i.e.* one scale completely encircles the circumference of the fibre and each scale sits in the cup formed by the outward and distally projecting edge of the one beneath it. As the fibre widens (Figs. 1 and 2) more than one scale per diameter is seen and these generally tend to stretch irregularly across the fibre with their length diagonally across the shaft, giving much



(*a*) Proximal portion.



(*b*) Distal portion.

Fig. 1. Wool. Surface markings. ×225.

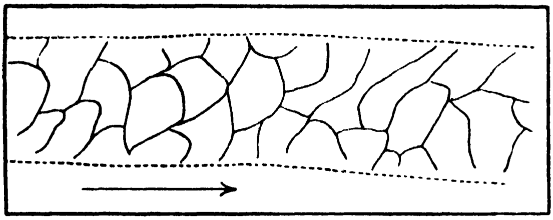
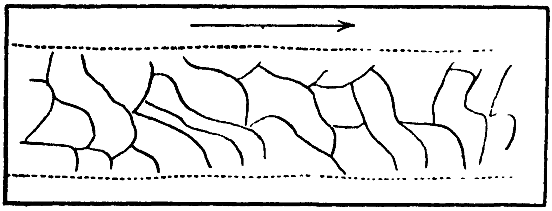
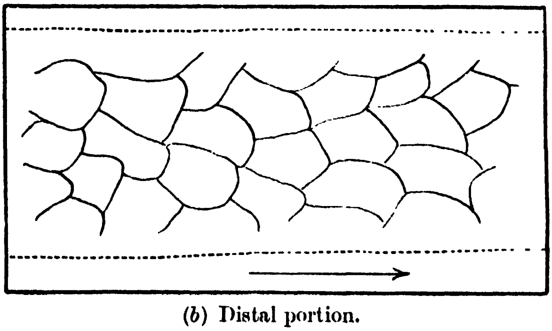


Fig. 2. Wool. Surface markings on thick wool fibre corresponding to Plate XVIII, fig. 4. ×225.

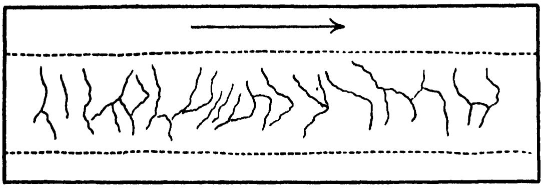


(*a*) Proximal portion.

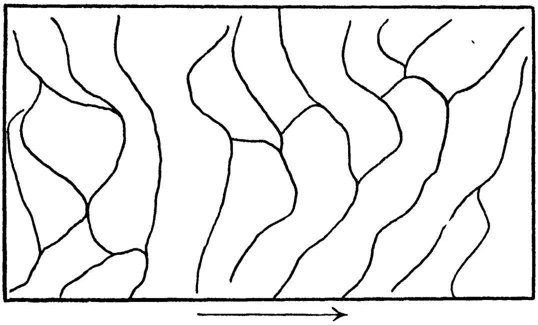


(*b*) Distal portion.

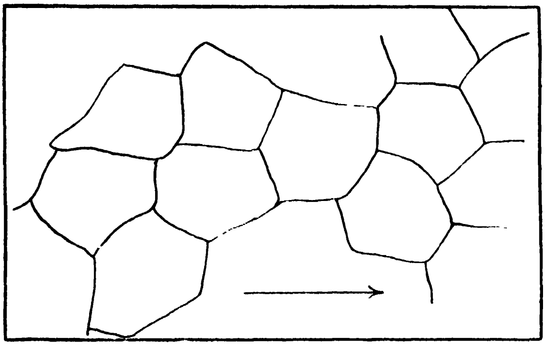
Fig. 3. Hair. Surface markings on the thick and thin portions of a hair fibre. ×225.



(*a*) Tip.



(*b*) Distal portion.



(*c*) Proximal portion.

Fig. 4. Kemp. Surface markings at different points along the kemp fibre. × 375.

the same appearance as is found in the finer portions of the hair type. As in the other types the tip is formed by the clear smooth cortex and the cuticular scales only, the medulla having terminated some distance from the end.

Structurely there is less to define the three classes of fibres found in the Blackface fleece than is given by the general appearance to the naked eye examination. The broad shallow scales (Fig. 4*b*) found on the distal half of kemp seem characteristic of that fibre and do not appear to occur in hair. Cortex and medulla on the other hand may be so similar in these two types as to be useless for their identification. In distinguishing microscopically between hair and wool the difficulty is still greater; here the cuticle lends no help and one can only say that in wool the proximal third of the fibre is finer whereas in hair only a small part of the proximal end is finer than the rest of the fibre.

Kemp appears to differ from the other two types of fibre in that it is completely shed at least once a year. From this, as from its structure, it seems that kemp is analogous to the hair of the primitive breeds. The "hair" of the Blackface is not hair defined on these standards but in its thicker parts approaches in structure the Intermediate (*a*) type of fibre found in *O. vignei*[1], while the wool varies from typical wool to something which tends to the Intermediate (*b*) type. Both these Blackface types are, of course, much longer than those of the primitive breeds and also differ in that only a few of their fibres are shed annually, the rest showing a finer part in this region which in the case of wool is continued distally a third of the length of the fibre. It may be noted that this "shedding" region corresponds to the "rise" of the fleece which is generally taken as the signal for clipping, so that actually the fleece is sheared at its weakest point.

The looping of the hair fibres which occurs in fleece (1) seems to indicate that the wool stops growing for a time while the hair held in check by the matt of fibres kept close above it by the non-lengthening wool fibres is forced to curl or bend in order to continue its growth. In this the Blackface fleece again shows a resemblance to the primitive breeds where there is no summer growth of wool. Following out this analogy it might be expected that there would also be a period of no growth in the hair and kemp fibres earlier in the season, but whether this occurs is not evident from the present material. "Grey" fibres appear to take their origin from pigmented patches in the skin, since all three types of fibre may be pigmented.

SUMMARY.

The Blackface Mountain sheep carries a very long and coarse fleece consisting of three types of fibre:

(1) *Kemp*, the shortest, coarsest fibre is completely shed annually. It has the structure of typical hair.

(2) *Hair*, the longest type, varies a great deal in diameter. Under normal domesticated conditions it has little or no tendency to curl. Only a small percentage of fibres are shed annually, the others showing a finer

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[1] F. A. E. Crew and J. S. S. Blyth. "On Certain Fibres of an Intermediate Nature found in *O. vignei*," *Ann. of Applied Biol.* x, p. 295.

part in the same region. In structure the coarser fibres resemble the Intermediate (*a*) type of *O. Vignei.*

(3) *Wool,* the finest fibre, tends to vary from typical wool structure to that of the Intermediate (*b*) type of *O. Vignei* and is finer in its proximal third than in its distal part. It is always curly more or less according as it is finer or thicker. Only a few fibres are shed annually.

The variation in Blackface fleeces would appear primarily due to the variation in the character of the second type of fibre. "Grey" fibres found scattered throughout the fleece may be of all three types.

Table I.

	Fleece (1)			Fleece (2)	
	Shoulder cm.	Britch cm.	Patch on right shoulder* cm.	Shoulder cm.	Britch cm.
Hair					
Average length of fibre	41·6	38·5	—	31·2	33·0
Longest fibre	43·9	41·1	—	32·0	35·1
Shortest fibre	37·3	29·5	—	30·2	29·2
Wool					
Average length of fibre	21·87	21·52	20·48	15·95	18·36
Average no. of waves/fibre	47 75	37 75	44·84	30·45	32 33
Average no. of waves/cm.	2 18	1·75	2 19	1 91	1·76
Greatest no. of waves/cm.	3·47	2 43	2 85	2 83	2·76
Longest fibre	30·5	25 9	22 6	20 82	22·86
Shortest fibre	18 55	17·78	18·04	11·7	14·0

* Patch containing practically no hair fibres.

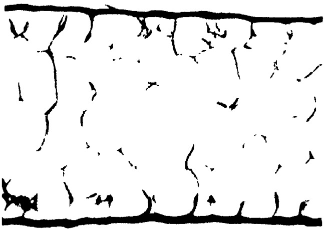
Table II. *Fleece* (1).

	Hair	Wool	Kemp*	Total	Total per sq. inch
	per ⅛ square inch				
Shoulder					
No. of fibres	315	960	74	1349	10,792
Percentage of fibres	23 35	71·16	5 49	—	—
Britch					
No. of fibres	417	751	109	1277	10,216
Percentage of fibres	32 65	58 81	8·54	—	—

* Only those kemp fibres which were attached to the skin were counted.

Table III. *Average Diameter.*

Fleece	Hair cm.	Wool cm.
1	·0076	·00277
2	·0058	·00307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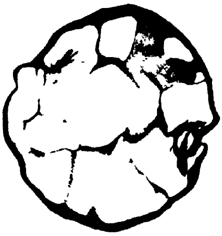
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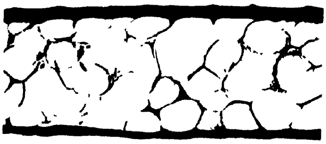
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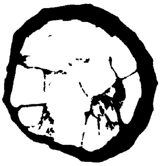
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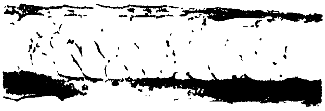
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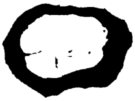
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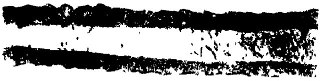
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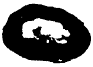
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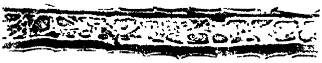
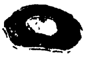

5 (*a*).


5 (*b*).

6 (*a*).

6 (*b*).

BLYTH.—Micrological Analysis of Two Fleeces from Blackface Sheep. (pp. 301—311.)

Table III (*a*).

Giving average diameters at different points along the fibres in both fleeces travelling from tip to root.

Fleece (1)	Fleece (2)
0·0094	0·0063
0·0086	0·0063
0·0061	0·0051
0·0071	—
0·0068	—

DESCRIPTION OF PLATE XVIII

Fig. 1. Kemp, thick fibre. × 200.

Fig. 2. Hair, thick part of medium sized fibre. × 200.

Fig. 3. Hair, thin portion of same fibre. × 200.

Fig. 4. Wool, distal part of one of the thickest fibres from the part of the fleece which was almost free from hair. × 200.

Fig. 5. Wool, distal portion. × 200.

Fig. 6. Wool, proximal portion. × 200.

a, sagittal section; *b*, transverse section.

(*Received June 6th*, 1923.)

CARBON DIOXIDE IN RELATION TO GLASSHOUSE CROPS

PART I.

THE DISTRIBUTION OF CARBON DIOXIDE IN GLASSHOUSE ATMOSPHERES

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(With 2 Text-figures.)

INTRODUCTION. Investigations carried out by continental workers during recent years have drawn attention to the possible increases in crop-yield resulting from the artificial enrichment of air with carbon dioxide. The results claimed have raised wide-spread interest in the horticultural world and in the interests of the glasshouse industry in this country it became essential to investigate how far the claims were valid under the conditions prevailing here.

Before attempting to increase the concentration of atmospheric carbon dioxide under glass it was imperative that some information should be available concerning its normal distribution and variation under working conditions. The work dealing with this phase of the problem is described in Part I.

It is generally held that in the open, atmospheric carbon dioxide is distributed in layers, the highest concentration lying near the soil surface. Under glass where manurial treatment is comparatively extravagant one would expect to find this state of things accentuated. It has been claimed that in a cucumber house "the concentration is greatest near the soil surface—nine to fourteen parts per 10,000—and least at the top of the house—four to five parts per 10,000" (1). Beyond this there seems to be no indication that glasshouse atmospheres have been analysed.

As will be shown here, no regular variation in the carbon dioxide concentration can be found at different levels in a commercial glasshouse. For the experiment a cucumber house measuring 100′ × 44′ was used, the other dimensions being shown in Fig. 1. Samples were taken at some or all of the points marked *a* to *m* and analysed.

It was found that the average concentration of carbon dioxide varies during twenty-four hours, showing a maximum in the early morning and a minimum in the early afternoon. An examination of Table I shows that the concentration at definite points varies very irregularly. As already stated, it was expected that a definite stratification of the gas would be found. If this were exactly so then the concentrations at the

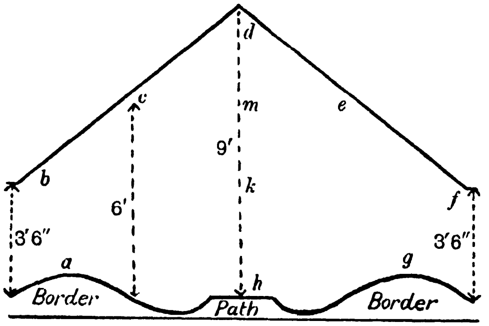

Fig. 1. Side elevation of house, showing the points at which samples were taken.

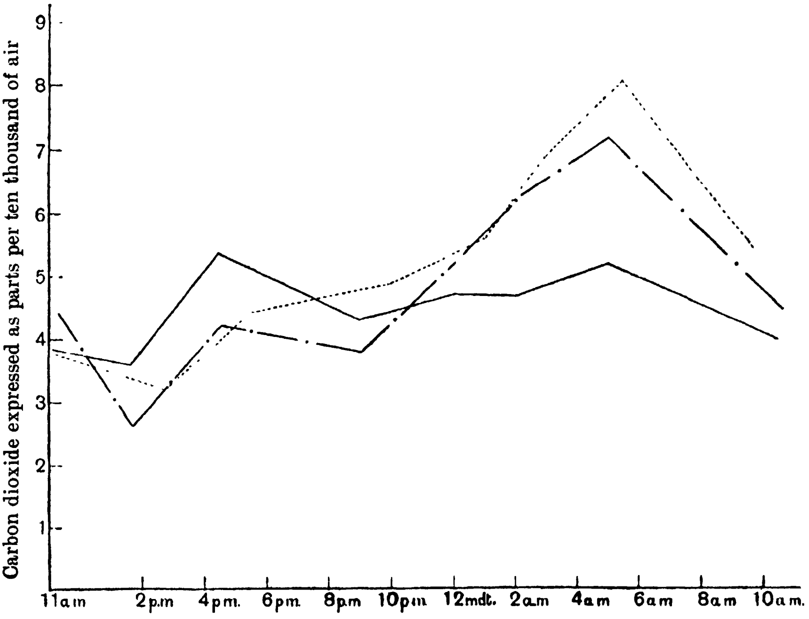

Fig. 2. Showing the variation, over 24 hours, in:

(1) the average concentration of carbon dioxide - - - - - - - - - - -

(2) the concentration at one of the soil surfaces ——• – – —•——

(3) the concentration at the apex of the house ——— ————

Table I. *Observations made during October* 1922[1].

		Position									
Date 1922	Time	*a*	*b*	*c*	*d*	*e*	*f*	*g*	*h*	*k*	*m*
Oct. 11	9.45 a.m.	3·4	1·5	—	5·8	10·0	7·6	9·0	3·6	6·5	2·7
,,	12.45 p.m.	1·1	3·7	1·8	3·7	1·5	—	4·5	3·0	4·4	5·7
,,	4 ,,	1·3	3·0	5·4	4·7	2·0	4·6	4·2	5·1	1·8	4·5
Oct. 12	11.30 a.m.	1·1	4·2	5·2	4·7	3·6	3·8	7·0	—	3·8	9·4
,,	3.30 p.m.	—	5·2	6·5	4·4	1·7	1·8	1·9	—	—	—
Oct. 13	9 a.m.	7·4	6·7	2·8	5·0	2·0	7·2	9·6	10·0	6·2	—
,,	5 p.m.	1·5	1·8	2·2	2·2	2·0	3·8	3·8	—	—	—
Oct. 16	9.30 ,,	1·2	5·5	2·4	—	9·5	4·7	5·1	6·3	6·8	6·9
,,	12 midnight	4·0	6·7	10·4	—	2·1	2·2	7·9	3·2	6·1	4·6
Oct. 17	3 a.m.	4·3	9·1	7·3	10·5	7·5	8·9	2·0	—	6·3	8·8
,,	6 ,,	3·0	5·7	8·8	8·4	3·0	5·1	7·2	16·1	3·4	5·3
,,	9.30 p.m.	5·1	5·8	4·0	6·3	11·9	4·6	5·3	2·5	3·9	8·0
,,	12 midnight	6·9	4·3	5·4	5·6	7·1	9·6	2·6	6·6	2·5	2·7
Oct. 18	3 a.m.	4·4	8·1	4·1	8·8	7·0	—	5·0	9·0	7·7	5·9
,,	6 ,,	—	6·7	8·1	8·4	6·2	8·8	5·2	7·1	5·0	7·0
,,	9.30 p.m.	5·3	4·0	7·0	3·8	3·8	8·0	5·7	5·9	7·8	4·4
,,	12 midnight	2·0	6·6	7·6	5·0	6·0	—	6·9	4·3	4·0	7·7
Oct. 20	3 a.m.	5·8	7·9	8·3	5·8	8·4	10·0	6·9	5·4	7·1	—
,,	11.15 ,,	3·0	2·7	3·6	4·0	—	—	—	4·6	4·0	5·9
,,	2.45 p.m.	3·8	2·9	2·5	3·6	—	—	—	2·7	4·0	3·5
,,	5.30 ,,	2·0	4·2	5·4	4·6	—	—	—	4·8	4·7	5·7
,,	10 ,,	4·9	5·4	4·7	4·4	—	—	—	3·8	—	4·8
Oct. 21	1 a.m.	5·5	4·4	—	4·9	—	—	—	5·1	7·4	5·7
,,	3 ,,	7·7	7·2	10·2	4·9	—	—	—	6·2	7·7	4·8
,,	5.30 ,,	7·7	8·1	11·2	6·2	—	—	—	7·4	8·8	7·1

positions *b*, *k* and *f* in Fig. 1 and also at *c*, *m*, and *e* would be approximately the same, but as will be seen from Table 1, no similarity occurs. In fact, individual variations are most irregular for all positions. While this is so, it is found that the average concentration varies comparatively regularly with time, this being shown in one of the curves in Fig. 2. The irregularities observed are difficult of explanation and at present one can only attribute them to the fact that the air in a glasshouse is never still. As a result, various currents must be set up and these would tend to disturb any localisation of carbon dioxide which might otherwise persist. In addition to this purely mechanical effect the processes of assimilation and respiration of the plants in the house come into play, although these may not produce the abrupt irregularities observed. It is also possible that the methods of sampling or analysis are at fault. Alternative methods of sampling were tried and will be dealt with presently. The actual analyses were carried out in the portable Haldane apparatus, and the Pettenkofer method seems to be the obvious alternative. This method however is hardly justifiable since the large volume of sample required would in itself be sufficient to cause quite an appreciable local disturbance.

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[1] Observations given as parts of CO_2 per 10,000 of air.

Since stratification could not be found under the conditions described it was considered desirable to determine whether it existed under different conditions. The soil of a small empty chamber of a thousand cubic feet capacity was covered with a layer six inches thick of fresh stable manure. It was thought that by this means the carbon dioxide in the air would be increased and any tendency on the part of the gas to stratify would be accentuated. Samples of the air were then taken periodically at heights of 1′ 3″, 3′ 6″, 7′ 6″ above the surface of the manure. Some dozen sets of observations were made, and typical readings are quoted in Table II. It is evident that there is no regular variation at different heights. In case the presence of growing plants might affect the atmospheric distribution the experiments just described were repeated in a cucumber chamber of similar dimensions in the presence of the same amount of manure. Typical results are shown in Table III. It will be observed that compared with Table II the results give lower values for the carbon dioxide. It would seem that this must be attributed to assimilation since all these determinations were made in the day time.

Table II. *The concentration of carbon dioxide in the atmosphere of an empty house containing only soil and fresh stable manure. Capacity approximately* 1000 *cu. ft.*

Height from soil surface	Concentration of carbon dioxide		
	10 a.m.	2 p.m.	4 p.m.
1′ 3″	11·7	5·5	3·2
3′ 6″	15·0	6·1	3·7
5′ 6″	14·7	4·7	3·5
7′ 6″	14·2	7·0	4·0

Table III. *Concentrations in a cucumber house with soil covered with a six inch layer of fresh stable manure. Capacity* 1000 *cu. ft.*

Height from soil surface	Concentration of carbon dioxide			
	10 a.m.	12 noon	2 p.m.	4 p.m.
1′ 3″	4·2	2·5	2·0	1·6
3′ 6″	3·6	2·4	2·4	1·5
5′ 6″	3·2	2·6	2·3	2·0
7′ 6″	3·2	2·5	2·3	2·0

It may be argued that the method of sampling might interfere with the sample taken. It was considered that in either of the two small chambers already referred to that this objection might be overcome by hanging the sample bottles on nails fixed at the different heights. This was done and the unstoppered bottles put in position about three hours before sampling. At the appointed time the bottles were quickly stoppered, taken out and analysed. Again similar results were obtained and are shown in Table IV.

Table IV. *Concentrations shown when sample bottles were left in position for three hours before analysis. Empty house* 1000 *cu. ft. capacity in which the soil was covered with a six inch layer of fresh stable manure.*

Height from soil surface	Concentration of carbon dioxide 10 a.m.	2 p.m.	4 p.m.
1′ 3″	6·1	5·4	5·0
3′ 6″	5·7	3·9	4·6
5′ 6″	6·3	4·4	4·8
7′ 6″	6·1	5·4	4·6

EXPERIMENTAL. The samples were taken in two-ounce bottles fitted with glass stoppers and were filled by aspiration, duplicates being taken simultaneously by means of a T-piece. The samples from positions *b*, *c*, *d*, *e* and *f* (Fig. 1) were taken above the foliage, while those at *a*, *g* and *h* were taken on the soil surface. They were all analysed in duplicate in the portable Haldane apparatus in the prescribed manner (2). In consequence the readings shown in the table are the mean of four determinations and all the results are expressed as parts of carbon dioxide per ten thousand of air.

ALTERNATIVE METHODS OF SAMPLING. The possibilities with an alternative method of sampling have also been examined. Apart from disturbances caused by the workers in a house, those due to the observer himself would tend to upset the readings. Accordingly it was thought that if samples could be taken from some distance away the difficulty would be overcome. An obvious suggestion is the arrangement of a system of glass tubing of sufficient length to obtain the desired sampling. To be effective the bore of the tubing must be small, otherwise displacement of air in sampling would be sufficient to cause an appreciable disturbance. About ninety feet of capillary tubing was fixed upon suitable supports—one end being open at the sampling position while the other was connected to the tap of a nitrometer. It was intended that mercury from the nitrometer should be forced down the capillary tubing till the latter was full. By altering the level of the mercury it would return and a sample should eventually be drawn into the closed limb of the nitrometer. The transference of the sample to the Haldane apparatus would then be a comparatively easy matter. Actually, in practice the whole procedure was found to be unsatisfactory. In spite of continual adjustments of the capillary tubing, filling it with mercury was invariably a lengthy and tedious process, and the taking of a sample was accomplished on very few occasions only. Air bubbles continually made their appearance and in spite of many adjustments it was impossible to obtain satisfactory results. After a fortnight this method was abandoned. The alternative was to take a sample from immediately outside the house by which means a much shorter length of capillary tubing would be necessary. This method was at once found to be unsatisfactory owing to the appreciable difference in temperature inside and outside a house.

In spite of the objections which have been noted to methods of sampling it is maintained that if the suggested atmospheric stratification does exist in glasshouses it would have been manifested in at least some of the many series of observations carried out in this work. It is concluded therefore, that such stratification does not exist.

CARBON DIOXIDE IN RELATION TO GLASSHOUSE CROPS

PART II.

THE PREPARATION OF AN ATMOSPHERE RICH IN CARBON DIOXIDE

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(With 3 Text-figures.)

Before proceeding to elaborate experiments upon the fertilising effects of atmospheric carbon dioxide it is necessary to be able to prepare at will, atmospheres rich in this gas. A review of recent literature on the subject and more especially on the use of waste gases from furnaces shows that while carbon dioxide has been passed into experimental houses, no estimation has been carried out of the concentration in the vicinity of the plants. In the present investigations an attempt has been made to evolve a method of obtaining a given concentration which will persist.

Preliminary experiments in a normal cucumber house showed that the carbon dioxide disappeared as soon as it was put in. Part of this was doubtless taken up by the plants, but it is probable that the greater part diffused through the ventilators, glass overlaps and door. Soil absorption might also occur. To eliminate these factors in the present work the soil was covered with a layer of concrete, the overlaps were filled with putty, and the door and all the ventilators of a small chamber well padded. The results obtained under these conditions form the greater part of the present communication.

Even with these precautions it was found that the carbon dioxide disappeared almost as quickly as it was introduced when it was allowed to diffuse *slowly* into the house. Furthermore, the distribution was irregular. It was decided therefore to investigate the effect of mechanical mixing by means of a fan. By this means complete distribution was obtained, but there was still a serious initial loss of gas, only one half of the amount introduced being found on analysis of the atmosphere.

A similar loss was found when an attempt was made to introduce a large volume of carbon dioxide in a short time by the action of sulphuric acid on sodium bicarbonate. In addition much delay was continually caused by the frothing of the mixture and consequently it always required a considerable time to add the requisite amount of acid. Later, using purer acid, it was found that comparatively little frothing took place and the addition of acid took much less time. In these circumstances as much as 98 per cent. of the theoretical amount of gas was found in the chamber. These results indicate that rapid generation of the theoretical amount of carbon dioxide and mixing by mechanical means are essential in the preparation of an atmosphere containing a given concentration of carbon dioxide.

With the precautions described above, a concentration of 30 parts per 10,000 can be maintained, but this involves firstly, the production of the initial concentration by means of bicarbonate and secondly, a continuous supply of carbon dioxide and continuous mixing by means of a fan. This method is, at best, cumbersome, and consequently for the projected experiments an alternative one was sought. The most promising solution lay in the determination of the rate of disappearance of the gas. By this means it could safely be said that, providing the rate of loss was a regular one, the concentration over a given time must lie between two limits, the limits being determined by experimental conditions. At the end of a given interval enough carbon dioxide could be introduced into the chamber to bring the concentration up to its initial value and hence the concentration throughout the day could be kept at any required *average* value.

Actually it was found that the rate of loss was quite regular and further, the regularity persisted even when the fan was used for the initial mixing only. To give therefore an even distribution circulation is only necessary during the decomposition of the bicarbonate. The relative effects are well brought out in the curves in Fig. 5.

It had been suggested in the early stages of the investigation that soil absorption played an important part in the loss of carbon dioxide, and to test this a layer of soil was placed on the floor of the house without being dried or treated in any way. The curves showing average rate of loss with and without soil in the house agree in a very striking manner as shown in Fig. 5. That obtained with soil in the house lies slightly below the other, but it is possible that this is to be explained by the relatively high humidity in the presence of soil. The relative humidity of the air seldom fell below 70 per cent. and averaged over 80 per cent.

The presence of about 70 plants of various ages in the house was without material effect on the rate of loss of carbon dioxide.

Finally it is worthy of note that even with the high concentration prevailing after stopping the fan no indications of stratification were observed.

EXPERIMENTAL DETAILS. Timmis (3) has already described the early work on this subject. The same work was repeated and amplified by one of the present authors (see Part I). Briefly the results obtained were as follows.

The experimental chamber used was at the north-east corner of a partitioned glasshouse. Its capacity was approximately 1000 cu. ft., so that one cubic foot of carbon dioxide introduced would correspond to a concentration of ten parts per 10,000.

While cucumbers were growing in the house, carbon dioxide from a cylinder was introduced through a meter and a perforated lead pipe. No steps were taken to ensure mixing, and under these conditions Timmis found that when ten cubic feet of carbon dioxide had been added, the concentration one hour later was ten parts carbon dioxide one foot above ground and seven parts eight feet above. A hole two inches deep in the soil showed a concentration of 64 parts carbon dioxide. From these results Timmis suggests that the main cause of the loss of the gas is by absorption in the soil.

EXPERIMENTS ON CIRCULATION. Early in the present year a fan with suitable driving mechanism was erected. The arrangement of the inlet and outlet tubes and of the carbon dioxide supply and sampling tubes is shown in Fig. 3. In the first experiments the soil was covered with a six inch layer of concrete. To obviate the necessity of entering the house to take samples, glass tubes were passed through the corridor wall and projected three feet in the house. They were closed by rubber tubing and screw clips. Fig. 4 shows their relative positions. Samples were taken at the ends *A*. The analyses were performed with the portable Haldane Apparatus and for the higher concentrations with the Large Haldane Apparatus (2). The analyses were done in duplicate and each result given is the mean of two or three readings.

CARBON DIOXIDE OBTAINED FROM A CYLINDER. After preliminary trials it was found that between two and three cubic feet of gas could be passed into the chamber in an hour, a greater rate tending to choke the apparatus by freezing.

The carbon dioxide was at first conveyed into the inlet of the fan which was kept running while the gas was being introduced. About one-

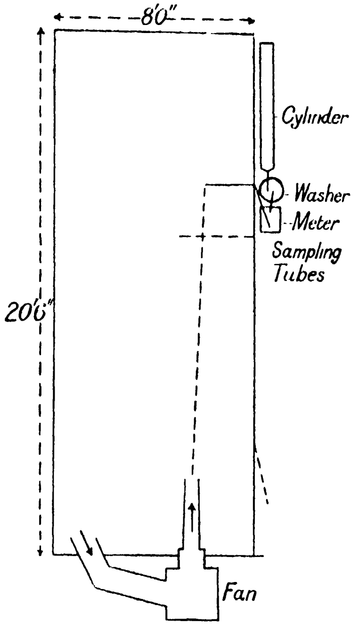

Fig. 3. Plan of chamber, showing fan, etc.

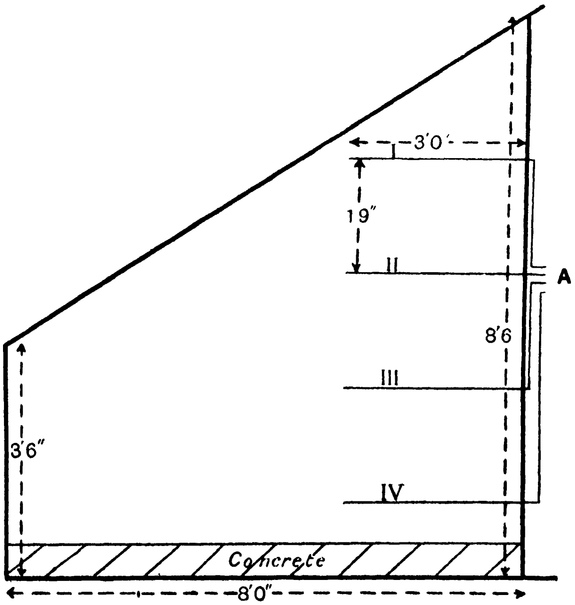

Fig. 4. Side elevation of chamber, showing sampling tubes (not to scale).

third of the carbon dioxide introduced could be registered and the distribution was complete. A set of typical results is given in Table V, the numerals referring to the sampling tubes shown in Fig. 3.

Table V. *Carbon dioxide from cylinder passed into inlet of fan.*

3·3 cu. ft. of carbon dioxide introduced should give 33·0 parts per 10,000.
Found:—15 minutes after gas was introduced.
I. 11·0 parts carbon dioxide
III. 10·7 „ „ „

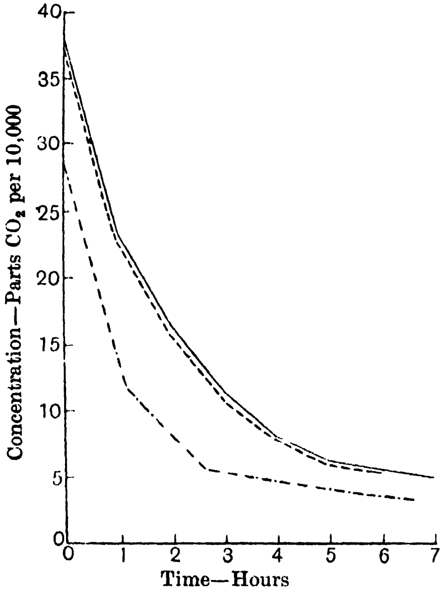

Fig. 5. Showing rate of loss of carbon dioxide:
(1) with continuous mixing ——•——•——•——
(2) mixing during introduction only—no soil —·—·— —·—·— —
(3) mixing during introduction only—with soil — — — — — — —

The carbon dioxide was now allowed to drip in front of the outlet of the fan, so that it was immediately dispersed about the chamber. The results are shown in Table VI.

Table VI. *Carbon dioxide from cylinder conveyed to outlet of fan.*

Theoretically 25·0 parts per 10,000.
Samples taken immediately:
Found: I. 14·2 parts carbon dioxide
II. 14·2 „ „ „
III. 13·9 „ „ „
IV. 13·7 „ „ „

Thus about half the carbon dioxide introduced could be found, and the distribution was complete.

These tables show the gas to be evenly distributed. Hence it is sufficient to take a sample through any one of the tubes as an indication of the average concentration at that time.

SODIUM BICARBONATE AS SOURCE OF CARBON DIOXIDE. To get an initial concentration higher than was possible by using a cylinder, sodium bicarbonate was decomposed by means of Rectified Oil of Vitriol. One and a half lbs. of bicarbonate were placed in a large pneumatic trough in the chamber and 30 per cent. acid was run in through sampling tube III until effervescence ceased. Theoretically 64·2 cu. ft. of the gas should be evolved from this weight of bicarbonate. Carbon dioxide was also passed in from the cylinder during the decomposition. About half the amount of carbon dioxide was found, *e.g.* 38·1 parts per 10,000 out of a theoretical 73·5 parts.

Using four and a half lbs. of bicarbonate, giving a concentration of 192-6 parts, 173 parts were found. The decrease in the initial loss is probably connected with the fact that three times the amount of bicarbonate was decomposed in only twice the time. With a purer acid the time was reduced still further and 193 parts were found. Similar results were obtained with smaller amounts of bicarbonate.

MAINTENANCE OF A GIVEN CONCENTRATION. The initial concentration was obtained by decomposing sodium bicarbonate, and carbon dioxide was then passed in from the cylinder. The supply of gas tends to fall off when it is allowed to evaporate rapidly owing to the cooling of the apparatus and hence the concentration gradually falls off.

Table VII. *Maintenance of a given concentration of carbon dioxide.*

Bicarbonate just decomposed	33·7 parts carbon dioxide
After 50 minutes	32·5 ,, ,, ,,
,, 1 hour 35 minutes	30·3 ,, ,, ,,
,, 2 hours 10 minutes	29·9 ,, ,, ,,

The concentration rapidly fell off as soon as the fan and supply of gas were stopped. Thus after 45 minutes it had fallen to 13·6 parts per 10,000.

DETERMINATION OF THE RATE OF LOSS OF CARBON DIOXIDE. The first experiments were designed to discover if stopping the fan as soon as the evolution of gas had ceased would affect the rate of loss. In one case the fan was kept running all day and samples were taken every ninety minutes. The rate of loss approximated very closely to that found when the fan was stopped as soon as the bicarbonate was decomposed.

Table VIII. *Fan running all day.*

Theoretically 64·2 parts carbon dioxide per 10,000

Found:

Bicarbonate just decomposed	28·5 parts	After 4 hours	4·7 parts
After 1 hour	11·7 ,,	,, 5½ ,,	3·6 ,,
,, 2½ hours	5·7 ,,	,, 6½ ,,	3·2 ,,

Table IX. *Fan stopped after decomposition of bicarbonate.*

Theoretically 73·5 parts carbon dioxide.

Found:

Bicarbonate just decomposed	38·1 parts	After 4 hours	7·7 parts
After 1 hour	23·2 ,,	,, 5 ,,	6·1 ,,
,, 2 hours	15·4 ,,	,, 6 ,,	5·7 ,,
,, 3 ,,	10·4 ,,	,, 7 ,,	5·1 ,,

EFFECT OF SOIL ON RATE OF LOSS. Virgin soil from the field and not dried in any way was placed in the house in a layer about four inches deep. The addition of soil greatly increases the relative humidity of the air but this does not appear to affect the rate of loss.

Table X. *Soil in the chamber.*

Theoretically 71·5 parts carbon dioxide per 10,000.

Found:

Bicarbonate just decomposed	37·1 parts	After 4 hours	7·1 parts
After 1 hour	21·6 ,,	,, 5 ,,	5·8 ,,
,, 2 hours	13·9 ,,	,, 6 ,,	5·8 ,,
,, 3 ,,	9·1 ,,		

Tables VIII, IX, and X are incorporated in Fig. 3.

Using four and a half lbs. of bicarbonate giving a concentration of 192·6 parts per 10,000, a similar type of curve was obtained although naturally it differed in slope.

Table XI.

Theoretically 192·6 parts carbon dioxide per 10,000.

Found:

Bicarbonate just decomposed	168·8 parts	After 4 hours	36·3 parts
After 1 hour	110·9 ,,	,, 5 ,,	26·1 ,,
,, 2 hours	73·7 ,,	,, 6 ,,	18·7 ,,
,, 3 ,,	51·5 ,,	,, 7 ,,	13·3 ,,

With a purer acid 193 parts were found out of a theoretical 197. Samples taken during the decomposition of the bicarbonate showed that the gas was evolved at the rate of nearly one cubic foot per minute.

SUMMARY OF PARTS I AND II.

(1) As a preliminary to the examination of carbon dioxide as a fertiliser under glass the distribution of the gas in the glasshouses has been examined.

(2) Contrary to general belief it is shown that the gas is not distributed in layers. The concentration has been in certain cases artificially increased but even then no evidence of stratification is found.

(3) It is also shown that the average maximum concentration in the presence of cucumber plants occurs between 4 a.m. and 7 a.m. and the minimum between noon and 3 p.m.

(4) A method is described whereby atmospheres containing up to 200 parts per 10,000 of air can be prepared.

(5) The gas is prepared from sulphuric acid and sodium bicarbonate, and, providing an initial mixing takes place, it is uniformly distributed.

(6) It is shown that the distribution is independent of:

(*a*) the total amount of carbon dioxide present,
(*b*) the presence of soil,
(*c*) ,, ,, ,, plants.

(7) A definite loss of carbon dioxide always occurs, and a curve showing the rate of fall has been obtained.

Among the factors which may be responsible for this disappearance are leaks, soil, wood, glass, manure, plants and moisture. The present paper shows that soil and plants are without effect, and it is proposed at the end of the present growing season to examine the effects of the other factors and if possible to account for the high losses.

REFERENCES.

(1) *Cheshunt Exp. and Res. Stat. 2nd Ann. Rep.* 1916, p. 9.
(2) HALDANE, *Methods of Air Analysis*, 3rd edition, London, 1920, p. 47.
(3) TIMMIS, L. B. (1922). *Cheshunt Exp. and Res. Station Ann Rep.* VIII.

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CORRELATION BETWEEN LOSS OF LEAF AND DAMAGE TO CROP IN LATE ATTACKS ON WHEAT

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(With 6 Text-figures.)

The following experiment was undertaken to find a basis for estimating the effect on the yield of wheat by obtaining the percentage of loss of leaf surface.

METHOD.

Two varieties of wheat were chosen for the test, namely, Little Joss and New Red. As soon as the ears began to unfurl one yard lengths in the drills were treated in the following ways:

(*a*) all leaf blades were cut off,
(*b*) half of each leaf blade was cut off,
(*c*) all leaves on the bottom half of the plants were removed.

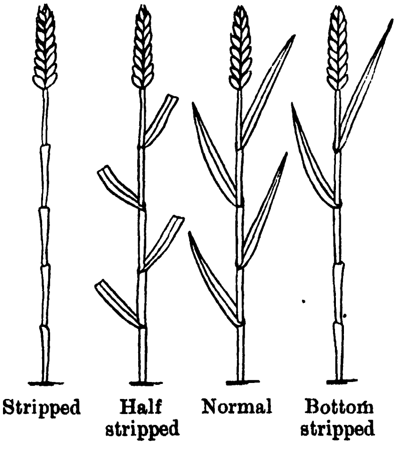

Fig. 1. Appearance of plants under test.

A series of three separate yard lengths of drills was obtained in this way on each variety.

The first plots were treated on July 2nd. A week later two other series, one on each variety, were similarly prepared. This was repeated each week until the crop was ripe and the leaves all withered. The last was prepared on August 15th making seven series in all. On the eighth week the crops were ripe and they were harvested on August 31st. In this way was obtained a succession of plots which had been badly damaged at regular intervals between flowering and ripening. The appearance of tillers under test is indicated in Fig. 1.

COMPILATION OF RESULTS.

There could obviously be no alteration in the number of ears produced because these were already formed when the test began. The effect of the damage could be on (*a*) size of ear, (*b*) size of grain, (*c*) a combination of these coupled with number of grains.

All the ears were collected on the yard drill lengths and the following measurements made: (1) average length of ear (average of ten on each yard length of drill), (2) average volume of 200 grains, (3) weight of grain on an average from ten ears.

For convenience the grains were rubbed out of ten ears and then weighed. No attempt was made to get in each case ten average ears, because all sound ears on the plot were weighed. The chief cause of the variation in the number of ears on the various yard drill lengths was damage by birds. All damaged ears were of necessity neglected.

For control and crop-average a number of groups of ten ears were obtained in different parts of the two fields and all averaged for the horizontal line of crop-average in each variety.

DATA.

A study of the lengths of ten ears (*vide* Table I) gave no indication whatever of interference with the leaf area. The table shows clearly the greater length of the Little Joss ear than that of New Red, the average length of the former being 11 cm. compared with 9·16 cm. in the latter.

Table II gives the volume in cubic centimetres of 200 grains. It will be seen that the grain from the stripped plant was in general smaller than that from the half-stripped plants on adjacent drills. In only three cases were they equal and in no case did the grain from stripped plants develop better than that from the half-stripped plants. The grain from the bottom-stripped plants was very variable and the average ultimately was the lowest. Although the figures are not sufficient to be of any use in determination of crop-loss it seems very clear that interference with the leaf area hindered considerably the filling out of the grain.

Table I. *Length of Ears.*

(10 ears in centimetres.)

Week	Stripped	Half-stripped	Bottom-stripped
Little Joss: Control 110·0			
1	108·5	96·1	103·3
2	—	—	—
3	104·4	119·2	101·5
4	104·8	101·2	103·2
5	92·7	109·9	106·5
6	105·4	107·1	102·1
7	105·5	107·8	104·3
New red: Control 91·6.			
1	88·7	78·6	82·8
2	93·1	85·6	83·8
3	87·0	85·6	86·8
4	87·1	82·2	89·5
5	89·9	91·0	89·4
6	92·4	94·3	96·4
7	91·8	—	—

Table II. *Volume of Grain.*

(c.c. per 200 grains.)

New Red: Control 7·5 c.c.

Weeks ...	1	2	3	4	5	6	7
Stripped ...	6·0	5·5	7·0	6·5	5·5	8·0	7·0
Half-stripped ...	7·0	6·5	7·0	8·0	8·0	8·5	7·0
Bottom stripped...	7·0	7·25	7·25	7·5	5·0	5·5	7·0

Little Joss: Control 7·1 c.c.

Weeks ...	1	2	3	4	5	6	7
Stripped ...	8·0	—	7·0	6·5	8·0	8·0	8·0
Half-stripped ...	8·0	—	7·5	7·25	6·75	7·5	8·0
Bottom-stripped...	7·25	—	5·5	6·5	7·75	7·0	8·25

Averages:

	Number used	
Control	1000	7·3 c.c. per 200
Stripped	2600	7·0 „ „
Half-stripped	2600	7·46 „ „
Bottom-stripped	2600	6·85 „ „

The figures for the weighings of grain from successive groups of ten ears are given in Tables III and IV for the varieties New Red and Little Joss respectively. Where more than two sound ears were left after grouping in tens these were weighed and the figures are given in brackets with the number weighed indicated outside the bracket in each case.

Table III.

New Red	Weight of grain from 10 ears in grams (except where in brackets)	Average weight of grain in 10 ears	No. of ears weighed
Control	23·65, 19·34, 18·55, 23·31, 20·35, 22·75, 24·30	21·75	70
1st week:			
Stripped	11·07, 10·65, 14·23	11·98	30
Half-stripped	6·35, 8·10, 7·08	7·20	30
Bottom-stripped	8·01, 9·50, 11·73, (8·45)^{8}	10·00	38
2nd week:			
Stripped	13·95, 15·92, 13·67, (10·38)^{7}	14·57	37
Half-stripped	15·04, 17·35, 21·67	18·02	30
Bottom-stripped	13·36, 13·24, 13·09, 19·37, (5·35)^{4}	14·64	44
3rd week:			
Stripped	14·78, 18·45, 14·95, 15·0, 17·02, 17·99	16·36	60
Half-stripped	21·24, 20·75	20·99	20
Bottom-stripped	22·32, 17·45, 17·91, 22·72, 21·27	20·33	50
4th week:			
Stripped	17·45, 15·57, 18·12, 20·58	17·93	40
Half-stripped	15·67, 15·75	15·71	20
Bottom-stripped	18·07, 14·79, 20·57, 23·68, (12·48)^{8}	18·66	48
5th week:			
Stripped	20·15, 16·42	18·28	20
Half-stripped	19·54, 19·42, 20·63, 17·95, (7·39)^{4}	19·30	44
Bottom-stripped	18·19, 14·96, (11·02)^{7}	16·36	27
6th week:			
Stripped	17·60, 18·47, 20·76	18·94	30
Half-stripped	22·60, 22·77, 22·38	22·58	30
Bottom-stripped	18·37, 13·64	16·01	20
7th week:			
Stripped	18·30, 21·45, 22·75, 20·10, 18·85, 19·88	20·22	60
Half-stripped	17·45, 15·57, 17·50, 18·71, (5·41)^{3}	17·36	43
Bottom-stripped	18·11, 22·20, 23·57, 21·34, 20·40, (9·77)^{5}	20·98	55
			846

Study of these figures reveals a regular and close connection between the damage to the leaf and the weight of grain obtained. The loss of crop was large when the plants were younger and became steadily smaller as the plant approached maturity. This is further and better shown in Curves 1 and 2.

In Table V and Curve 3 the figures for the two varieties are combined to lessen varietal influence. These show more clearly the close connection between loss of crop and damage to leaf area. It seems clear that weight of grain from ten ears is a good unit for the purpose of this experiment combining as it does the relative plumpness of the grain with the number of grains produced.

Table IV.

Little Joss	Weight of grain from 10 ears in grams (except those in brackets)	Average weight of grain in 10 ears	No. of ears weighed
Control	20·99, 18·50, 16·32, 20·22, 19·47, 17·47, 18·47, 15·50, 22·45, 19·50	18·89	100
1st week:			
Stripped	11·91, 12·52, 9·46, 10·89	11·20	40
Half-stripped	14·04, 14·85, 16·60, 12·35, 9·22, 14·15	13·54	60
Bottom-stripped	12·07, 16·25, 12·11, 15·70, (6·40)^{4}, 11·95	13·79	54
2nd week:			
Stripped	(11·71)		
Half-stripped	(15·16)		
Bottom-stripped	(14·06)		
3rd week:			
Stripped	10·72, 15·10, 11·94, 11·92, (4·12)^{4}	12·23	44
Half-stripped	16·81, 17·21, 18·54, 13·96, 18·60, (10·59)^{7}	16·79	57
Bottom-stripped	15·84, 13·30, 13·89	14·34	30
4th week:			
Stripped	11·57, 12·65, 11·85, (7·8)^{5}	12·53	35
Half-stripped	18·05, 17·10, 16·35	17·17	30
Bottom-stripped	16·16, 12·47	14·32	20
5th week:			
Stripped	12·45, 12·75	12·60	20
Half-stripped	14·95, 14·95, 12·56, 15·52	14·50	40
Bottom-stripped	17·61, 16·76, 13·47, 11·07, 14·90	14·75	50
6th week:			
Stripped	17·37, 18·17, 15·15	17·46	30
Half-stripped	17·92, 17·98, 14·44	16·78	30
Bottom-stripped	19·97, 20·45, 12·7, 11·3, 19·72	16·83	50
7th week:			
Stripped	20·94, 18·47, 19·17, 17·85, 17·01, 17·7, (14·20)^{9}	18·16	69
Half-stripped	18·98, 21·17, 18·09, (8·15)^{5}	18·97	35
Bottom-stripped	19·86, 15·26, 18·35, 17·62, (10·39)^{6}	17·71	46
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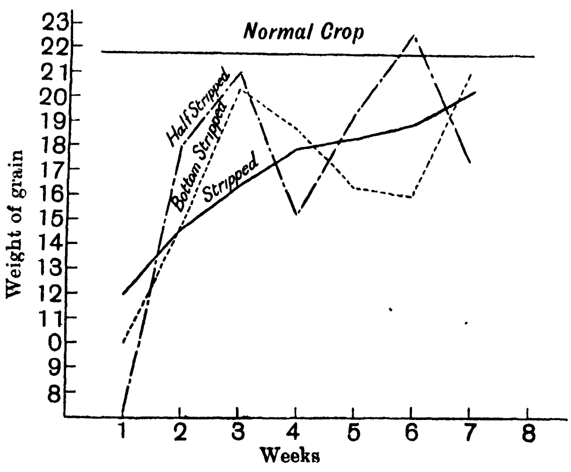
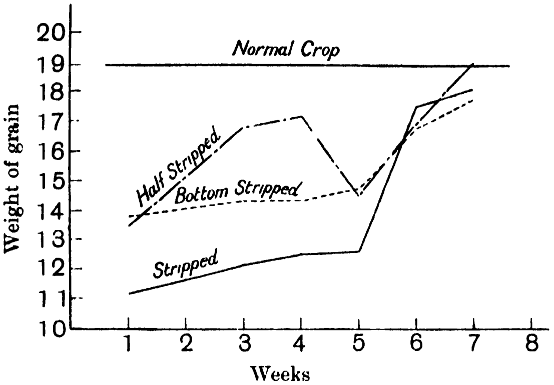


Curve 1. Variety, New Red.



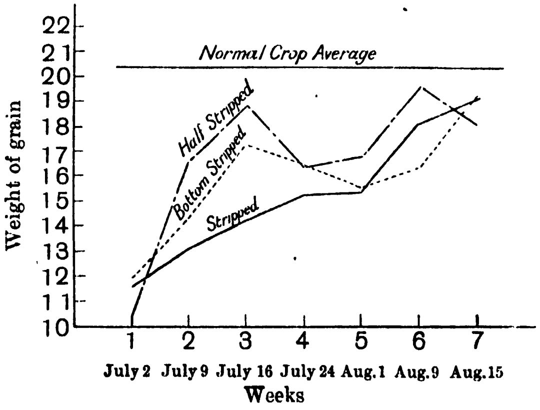
Curve 2. Variety, Little Joss.

Table V. *Figures for Little Joss and New Red combined.*

(Average weight of grain rubbed from 10 ears, in grams.)

Control: Little Joss 18·89; Now Red 21·75; Combined 20·32.

Weeks ...	1	2	3	4	5	6	7
Stripped:							
Little Joss	11·20	11·71	12·23	12·53	12·60	17·46	18·16
New Red	11·98	14·57	16·36	17·93	18·28	18·94	20·22
Combined	11·59	13·14	14·29	15·23	15·44	18·20	19·19
Half-stripped:							
Little Joss	13·54	15·16	16·79	17·17	14·50	16·78	18·97
New Red	7·20	18·02	20·99	15·71	19·30	22·58	17·36
Combined	10·37	16·59	18·89	16·44	16·90	19·68	18·16
Bottom-stripped:							
Little Joss	13·79	14·06	14·34	14·32	14·75	16·83	17·71
New Red	10·00	14·64	20·33	18·66	16·36	16·01	20·98
Combined	11·89	14·35	17·33	16·49	15·55	16·42	19·34



Curve 3. Combined Curve for two varieties.

GENERAL NOTES AND DEDUCTIONS.

The crops were rather late in flowering and fertilisation took place during the first week of July. On the 8th week, August 22nd, the leaves had all withered so no plots were made. Harvest took place on August 31st, the produce on the plots being taken indoors at once.

Interference with the leaves on such a scale as in this experiment produced a very serious alteration of crops during the period of fertilisation, approximately reducing it 50 per cent. in all three cases and in both varieties. This great depreciation of the crop and crossing of the curves seems to suggest a physiological disturbance. It seems undesirable to include the period of fertilisation in the rectification of the curves until it has been further studied and for this reason it is omitted in such rectification given below.

The bottom stripped plants consistently cropped low, suggesting that the lower leaves play a considerable part in grain formation. These leaves are larger than the upper ones so that the crop would be between the Stripped and Half-stripped plants assuming all leaves to have equal value for grain formation.

The Control crop averages bear out the superiority of New Red over Little Joss for the district (Shropshire). This is in accordance with several years' trials.

The greater irregularity of the New Red curve is partly due to its being a variety specially suited to the locality and therefore better able to take advantage of soil, manures, etc.

The drop in the curve for Little Joss in the 5th week was due to an attack of Yellow Rust. As this was on the lower leaves it did not appreciably affect the Stripped or Bottom-stripped plants.

There is a general upward trend in the curves from the 2nd to the 7th week and a study of the average figures for the crop depressions shows a very complete correlation between leaf blade loss and diminished crop. This seems to justify rectification of the curves for statistical purposes in recording crop loss by measurement of leaf loss.

Straightened Curves for Stripped and Half-Stripped Plants.

Curve 4 gives the mean straight curve prepared from Curve 3.

Line *A* is the curve for the Stripped plant. This shows the gradual diminution of the crop depression from the yield 12·76 in the 2nd week to reaching full crop 20·32 of the untouched plant at 8th week.

Line *B* is obtained by halving the crop alterations to correspond to half-stripping.

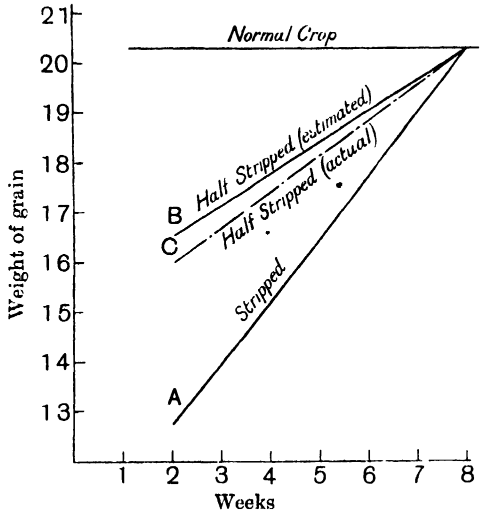
Line *C* is the straightened curve from the results actually obtained in the field and corresponds very closely to line *B* obtained as above indicated.

From these Curves *A* and *B* the total crop depression at the various weekly intervals can be stated, thus:

Table VI.

	Percentage crop obtained	
	Stripped	Half-stripped
2nd week	62·8	81·4
3rd ,,	69·0	84·5
4th ,,	75·2	87·6
5th ,,	81·4	90·7
6th ,,	87·6	93·8
7th ,,	93·8	96·9
8th ,,	100·0	100·0

This provides a basis for calculating the effect on the cereal yield of various leaf-attacking pests.



Curve 4. Straightened Curves for Crop Depression.

Percentage Alteration of Crop during the test.

A. During Fertilisation (1st week):

Normal Crop 3 × (average) = 3 × 20·32 = *60·96*

Depressed crop $\left\{\begin{matrix}\text{(Stripped)}\\ 11{\cdot}59\end{matrix}\right\} + \left\{\begin{matrix}\text{(Half-stripped)}\\ 10{\cdot}37\end{matrix}\right\} + \left\{\begin{matrix}\text{(Bottom-stripped)}\\ 11{\cdot}89\end{matrix}\right\}$

= *33·85*

i.e. Normal Crop ... ... 100 %

Crop obtained ... ... 55·5 %

B. Average depression during the next six weeks:

Normal crop 6 × 20·32 = *121·92*
Crop from stripped plants:
= 13·14 + 14·29 + 15·23 + 15·44 + 18·20 + 19·19 = *95·49*
Crop from half-stripped plants:
= 16·59 + 18·89 + 16·44 + 16·90 + 19·68 + 18·16 = *106·66*
Crop from bottom-stripped plants:
= 14·35 + 17·33 + 16·49 + 15·55 + 16·42 + 19·34 = *99·48*

i.e.

Normal crop	... ...	100 %
Stripped crop	... ...	78·3 %
Half-stripped crop	...	87·5 %
Bottom-stripped crop	...	81·6 %

These figures further illustrate the regularity of crop depression due to loss of leaf surface. They are represented in diagrammatic form in Fig. 2.

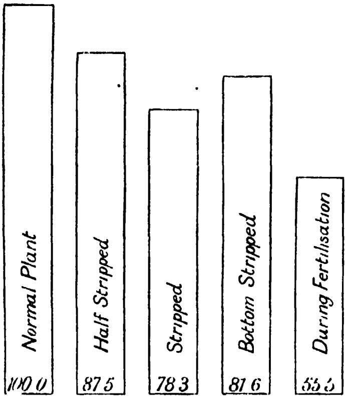


Fig. 2.

Use of Table VI or Curve 4.

An attack of *Agromyza ambigua* was observed on Marshal Foch wheat during 1922. Area 19 acres. Yield 48 bushels per acre. The attack was around 3rd to 7th weeks.

Percentage of attacked plants	...	10
Leaf surface of plant lost	...	·2
Leaf surface of field lost	...	2 %
Depression for total loss of leaf	100 − 81·4 =	18·6 %
Depression for 2% loss of leaf	$\frac{18·6}{50}$ =	·37 %
Yield obtained = 912 bushels per 19 acres	=	99·63 % full crop
Full crop should have been	...	915·5 bushels,
or the loss due to this pest was	...	3·5 ,,

This is used in illustration purely. Such great accuracy is never likely to be obtained or required but it serves to classify this pest as one of the lesser evils of the wheat crop although it looked much worse in the field.

(*Received January* 10*th*, 1923.)

THE KILLING OF *BOTRYTIS CINEREA* BY HEAT, WITH A NOTE ON THE DETERMINATION OF TEMPERATURE COEFFICIENTS

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(With 5 Text-figures.)

In a previous paper (1) some results were given of a study of the action of phenol on the spores of *Botrytis cinerea*. The present communication contains the results of a similar enquiry into the action of moist heat. The method employed was essentially the same as that used in the phenol experiments, and consists in dropping spores of the organism, grown always under the same conditions and for approximately the same time, into distilled water of the temperature under investigation, removing samples from time to time, and determining for each sample the proportion of spores able to germinate on Czapek's agar at a temperature of 24–25° C. The strain used was the same as that previously described.

In determining the proportion of germinable spores a difficulty was experienced which was not met in the phenol work. In the latter case little or no delay was found in the germination of spores capable of development, practically all the viable spores germinating within 24 hours. This however is not the case after exposure to heat. While most of the germinable spores do put out their germ-tubes within 24 hours of transference to the nutrient medium, there is regularly a significant proportion which delays doing so for a period that is longer or shorter according to the temperature to which the spores have been exposed and the duration of the exposure. For example, after an exposure of three hours to 37° C. a sample was transferred to Czapek's agar and counted after 24 hours' incubation at 24–25° C.: out of 710 spores 49·8 per cent. were found to have germinated. After a further 24 hours' incubation the same slides recounted showed 74·43 per cent. of the spores to have germinated; and after 24 hours more the number was found to be 68·2 per cent. The fall in the last count is experimental and illustrates the difficulty of accurate observation in a preparation which after 72 hours' incubation has become greatly overgrown. The hyphae from the spores which germinate early form appressoria and cover over many spores, so that at the last count, for example, only 324 spores could be counted out of the 710 known to be present in the preparations. Again, after 6 hours' exposure to a temperature of 37° C. the numbers germinated after a further 24 hours at 24°–25° were 2·3 per cent. out of 1300 present; 3·9 per cent. after 48 hours and 6·6 per cent. after 72 hours, after which time no further increase took place. With short exposures, on the other hand, *e.g.* $1\frac{1}{2}$ hours at 37°, the number germinated in 24 hours does not increase on further incubation. This delay indicates that the killing is a gradual process, and that the spore may be considerably damaged, may be in fact "half-dead," and yet recover sufficiently after a time to resume its normal course of development. It was not determined whether these late-developing spores showed other signs of altered vitality but no such alteration was observed. Weiss (2) has noted a similar effect of heat on the spores of *Bacillus botulinus*—the longer the period of heating before death occurs, the longer the period necessary for the subsequent germination of the spores.

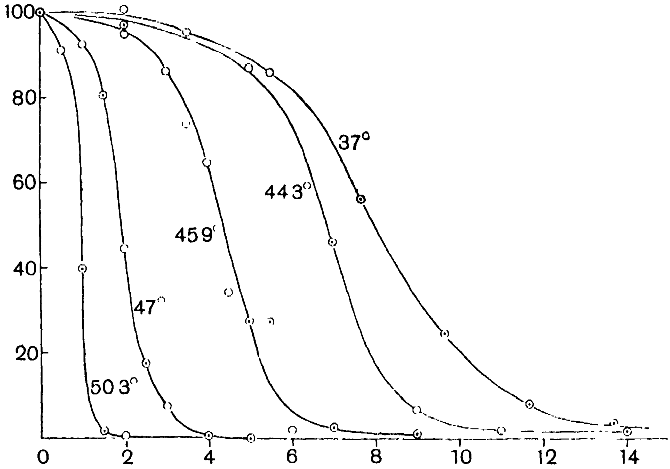

Fig. 1. Percentage of spores surviving plotted against time (abscissae). The time-scale represents minutes for the four higher temperatures; and half-hours for the 37° curve.

The results obtained are shown in Tables I—V, and the data are charted in Fig 1. In this graph the abscissae represent time from the start of the experiments, the ordinates the percentage of survivors. The time scale for the four higher temperatures is in minutes but for the results at a temperature of 37° C. it represents half-hours. It will be seen that the process of killing falls into two stages, a stage during which progressively increasing numbers of spores are killed in successive units of time, and a stage in which progressively diminishing numbers are killed. The curve is sigmoid in shape, with the point of inflection at the time when the maximum number of spores is being killed, and the two halves of each curve are approximately symmetrical.

Table I.

Temp. 37° C. 400,000 spores in 1 c.c. Control: 87·09 % germinated.

Sample taken in *mins.*	Number counted	Percent. germinated	Percent. calculated*
0	984	100·0	100·0
60	1150	100·4	98·0
106	1217	94·62	94·5
182	710	85·46	86·6
230	814	55·86	56·3
290	1076	24·41	24·5
352	1657	7·96	7·9
410	987	3·6	2·7
1380	1500	0·0	0·0

* See p. 341.

Table II.

Temp. 44·3° C. 440,000 spores in 1 c.c.
Control: 92·57 % germinated.

Sample taken in *mins.*	Number counted	Percent. germinated
0	1750	100·0
2	1314	96·2
5	1249	86·5
7	695	45·8
9	896	6·38
11	997	1·72
14	1261	1·44
20	1267	0·24
40	1600	0·129

Table III.

Temp. 45·9° C. 494,000 spores in 1 c.c.
Control: 95·30 % germinated.

Sample taken in *secs.*	Number counted	Percent. germinated
0	897	100·0
120	770	95·3
180	650	86·0
210	782	73·4
240	1003	64·5
270	1163	34·0
300	654	27·3
330	1262	27·02
360	1523	1·99
420	1659	2·46
450	1603	0·85

Table IV.

Temp. 47° C. 570,000 spores in 1 c.c.
Control: 92·74 % germinated.

Sample taken in *secs.*	Number counted	Percent. germinated
0	964	100·0
62	1237	92·3
92	1179	80·3
122	1396	44·4
152	1099	17·5
182	1761	7·22
242	1267	0·603
302	1214	0·0

Table V.

Temp. 50·3° C. 930,000 spores in 1 c.c.
Control: 97·24 % germinated.

Sample taken in *secs.*	Number counted	Percent. germinated
0	1595	100·0
30	1757	91·0
60	1528	39·8
90	1839	1·33
120	1806	0·34
240	1834	0·17
300	1900	0·05
360	1900	0·0

The series of curves bears a general resemblance to that obtained with phenol used in increasing strengths (1), but there is a difference in the two sets which points to a difference in the mode of action of the two killing agents. In the case of phenol the shape of the curve gradually changes as the strength of the poison is increased, the sigmoid character tending gradually more and more to disappear owing to the flattening out of the first elbow or bend in the curve. Eventually with a sufficiently high dose of phenol the sigmoid shape is completely lost and is replaced by a logarithmic type of curve, corresponding to that of a unimolecular chemical reaction. With heat as the killing agent there is no tendency for the curve to change into the logarithmic type. The curve remains the same throughout, and the process runs alike at all the temperatures, the only difference being in the rate at which it occurs.

This may be seen by comparing the times required to kill 25 per cent., 50 per cent. and 75 per cent. of the spores at the different temperatures with the times required at 50·3° C.

Table VI.

	Time in mins. required to kill			Ratio of the time at each temp. to the time at 50·3°		
	25 %	50 %	75 %	25 %	50 %	75 %
31°	9240·0	12140·0	—	11922·0	13202·0	—
37°	199·0	240·0	286·0	256·0	261·0	263·0
44·3°	5·8	6·8	7·84	7·4	7·4	7·2
45·9°	3·56	4·32	5·0	4·5	4·7	4·5
47°	1·65	2·0	2·33	2·1	2·2	2·1
50·3°	0·775	0·918	1·087	1·0	1·0	1·0

As Table VI shows, the time ratios at each temperature remain practically the same for all the stages of the process, which would not be the case if there were any significant change in the shape of the curves.

The same fact is perhaps even more clearly shown in Fig. 2, where all the observations at all the temperatures are plotted on the one diagram. The curve is drawn directly from the formula of Student's curve (3), discussed later, the time to kill 50 per cent. of the spores being taken as 1 on the abscissa line, and the ordinates representing the proportion of spores killed. For each of the temperatures studied, the time found to be necessary to kill 50 per cent. of the spores is similarly taken as 1, and the observed results entered on the figure correspondingly. For example, at 37° C. the time required to kill 50 per cent. of the spores is 240 minutes, which is taken = 1 on the time scale. The observed time of 106 minutes therefore corresponds to 106/240 = ·442 on the figure, and at this time 94·62 per cent. spores were found surviving, *i.e.* 5·38 per cent. had been killed. It is evident that all the observations at all the temperatures are arranged closely along the curve, although at 37° C. the process requires over eight hours for completion and at 50·3° C. only three to four minutes. The relative susceptibility of the individual spores remains the same in spite of the very great increase in activity of the killing agent.

As we have seen, this was not the case with phenol. A spore which was five times as resistant as another to a low strength of phenol was not five but more than five times as resistant to a high strength. In the previous paper this change was attributed to an alteration in the relative permeability of the individual spores as the poison was increased in strength. There are, we may suppose, two stages necessary in the process of killing of any spore by phenol. There is, first, the stage of penetration, during which the poison is making its way through the external coat or wall of the spore and, secondly, the stage of coagulant or other action on the spore contents after penetration has occurred. It was to a change in the relative rapidity with which the first stage was effected that the change in curve-shape was attributed; and this view is supported by the absence of the change in the heat experiments, because with heat the first stage is virtually eliminated. The *Botrytis* spore, a small body measuring $12\mu - 16\mu$ in its longest axis, takes, with only

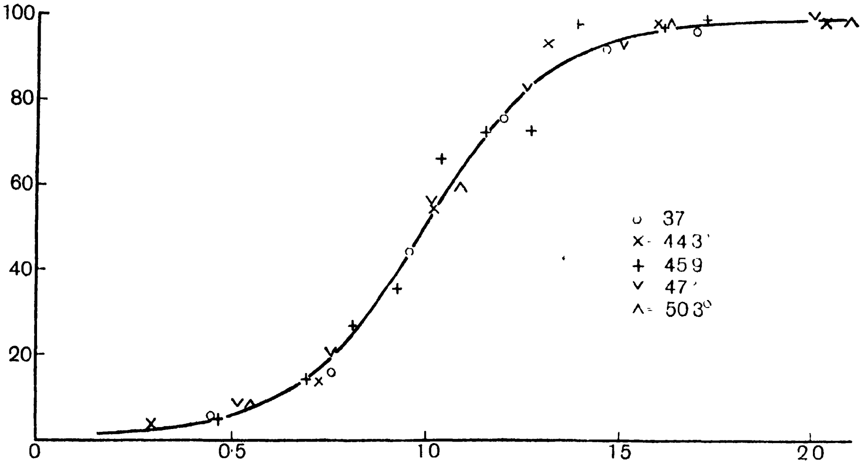

Fig. 2. The observations at five temperatures plotted on the one chart. The curve is drawn from Table XXV in *Tables for Statisticians and Biometricians*, with $n = 5$. The abscissae represent times, the time required to kill 50 % of the spores being taken as 1 for each temperature. The ordinates represent percentage of spores killed.

a slight delay, the temperature of the water in which it is suspended and the difference in time between the death of two spores of the same suspension (a difference, it may be, of hours) can scarcely be due to any significant extent to a delay in the heating of the spore which survives the longer. The differences in the times of death are due to differences in resistance of the spore-contents to the action of heat. This action which, as we have seen, proceeds gradually, may take long periods to reach completion and allows of the occurrence of the delayed germination already described.

The Shape of the Curve. The shape of the curve at once suggests a frequency distribution of resistances to killing among the individual spores, and this seems the most reasonable of the explanations that might be offered. Inspection suggested that the distribution would be nearly normal, and this apparent normality was tested by expressing each time interval as a fraction of the time taken to kill 50 per cent., and plotting these as abscissae against the percentages killed, expressed as deviates of the normal curve. The results of all five temperatures were plotted on one diagram; and if the proportions dying at the various times were normally distributed, these points would approximate to a straight line. There was however a distinct bending away in opposite directions at either end of the range, indicating that the distribution is not fully described by the normal curve but would be better represented by a curve such as that investigated by Student (3). Using Table XXV, given in *Tables for Statisticians and Biometricians* (4), with $n = 5$, and plotting the values of z against the time scale as before, the points were found to be closely grouped along a straight line. There was no tendency to an S-shape although a slight scatter occurred at the longer exposures (possibly due to post-mortem leaking of the contents of the spores first killed). At the five temperatures examined, therefore, the distribution of susceptibility to death at successive intervals of time is well described by Student's curve, of which an illustration is given in Fig. 3 to show the

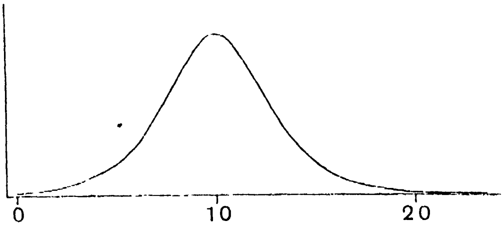

Fig. 3 Curve showing the distribution of spore-resistances.

type of distribution. This curve was used in drawing the curve in Fig. 2, where it will be seen that it fits the observations at all the temperatures very well indeed, especially when we consider that the experiments charted were carried out over a period of some months. With it we can represent with considerable accuracy the course of the reaction at any temperature within the limits examined, if the time required to kill 50 per cent. of the spores is known. In the last column of Table I are set out alongside the observed values the calculated values to be expected at the times indicated and it will be seen that the agreement is very satisfactory.

Temperature Coefficients. From Table VI (*supra*, p. 338), in which are given the times required to kill 50 per cent. of the spores at temperatures ranging from 31° C. to 50·3° C., it is evident that the influence of temperature on the rate of the reaction is remarkably great. At 31° C. the time is 12,140 minutes, at 50·3° C. it is only 0·92 minute, *i.e.* a rise of temperature of 19° C. has accelerated the reaction 13,200 times. The rise from 37° C. to 47° C. increased the velocity 120 times. Further, the temperature coefficient varies rapidly throughout the range of the temperatures examined. In Table VII are set out the coefficients for 1° C. rise (Q 1) over the intervals named, and the corresponding values for 10° C. (Q 10), if the same rate of increase had been maintained.

Table VII.

	Q 1	Q 10
31° -37°	1·92	690·0
37° -44·3°	1·63	132·0
37° -47°	1·61	120·0
44·3°-47°	1·57	92·8
47° -50·3°	1·40	29·5

In most chemical reactions the effect of a rise of 10° C. is to increase the velocity two or three times, so these coefficients in the case of *Botrytis* and moist heat are enormously high. There are however instances in the literature where similarly high coefficients have been obtained with similar material and heat; *e.g.* with agglutinins (5), bacterial toxins and other antigens (6) a coefficient of 2 for 1° C. rise (1024 for 10° C.); crystalline egg-albumen (7), 1·91 for 1° C. (646 for 10° C.); *Bacillus typhosus* (8), 1·63 for 1° C. (136 for 10° C.); *staphylococcus* (8), 1·4 for 1° C. (29 for 10° C.); *crystalline haemoglobin* (7), 1·3 for 1° C. (13·8 for 10° C.); *Bacillus coli* (8), 1·28 for 1° C. (12 for 10° C.).

It is perhaps scarcely to be expected that the relationship between temperature and velocity of the reaction found in these experiments should be adequately expressed by the formulae of van t' Hoff or of Arrhenius. These formulae are empirical and were devised to meet the case of chemical reactions in which the velocity changes slowly over a long range of temperature, and not such a case as the present where the velocity is changing very greatly over a short range. It is however a remarkable fact that the formula of Arrhenius does express the relationship with great exactness, if we reckon the temperature, not from the absolute temperature of – 273°, as Arrhenius does, but from 0° C. The values of the expression $\frac{T_0 T_n}{T_0 - T_n} \log \frac{t_n}{t_0}$ are given in the following table, in which the temperature T_0 is taken as 37° C. and the time t_0 as 240, the 37° C. experiment being probably the best observed of the series.

Table VIII.

	$\frac{T_0 T_n}{T_0 - T_n} \log \frac{t_n}{t_0}$
31°	325·6
37°	—
44·3°	347·5
45·9°	334·8
47°	361·5
50·3°	338·3
Mean:	341·5

The results show a very good approach to constancy. Further, if $\frac{T_0 T_n}{T_0 - T_n} \log \frac{t_n}{t_0} = k$, then $\log t_n = \frac{K}{T_n} - \left(\frac{K}{T_0} - \log t_0\right) = \frac{K}{T_n} - A$, a constant, since t_0 and T_0 are known and constant; and the values of $\log t_n$, plotted against $\frac{K}{T_n}$, or the reciprocal of T_n, should lie on a straight line. This has been done in Fig. 4, and it will be seen that the observations at all the temperatures do fall upon a straight line.

This expression, then, enables us to deduce the time that would be required to kill 50 per cent. of the spores at any temperature within the range of 31° C.-50° C.; and, by the aid of the other formula for the curve already given, the whole curve of the process of the killing can be drawn for any temperature within these limits. The whole process is, in fact, expressed by these two formulae for the spores of *Botrytis cinerea*, of the strain and under the conditions used in these experiments.

It is of interest to consider what is to be expected at temperatures below 31° C., *i.e.* on prolonging the line of Fig. 4 upwards. At 31° C. the temperature of the last observation on the figure, one is on the confines of the zone where death ceases and growth occurs. In distilled water at this temperature the organism dies: in a good nutrient medium it germinates and grows sparingly for a time. At 25° C. it grows well in a nutrient medium, and even in distilled water it germinates and grows freely for a time if it can obtain sufficient oxygen. At 25° C. in distilled water it dies if the oxygen supply is insufficient. If the spores are sunk in water to a depth of six inches, they do not receive enough oxygen for their development, and under these conditions they do not simply remain alive and inactive but die very slowly. After 37 days at this temperature 23·5 per cent. of the spores are already dead—a figure which, though greater than the expectation, is not so far removed from it but that the death may reasonably be attributed in large measure to the action of the temperature. Death from heat occurs even at temperatures very suitable for growth if a necessary condition of the growth be withheld. In fact one might say that all the spores would eventually be killed even at 25° C. and probably lower temperatures by the heat, if it were not for the occurrence of the opposing process of growth. Further consideration of this important question, however, must be postponed.

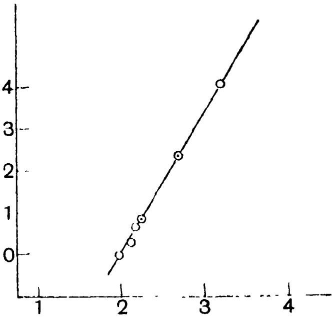

Fig. 4. The logarithms of the times taken to kill 50 °/$_{\circ}$ of the spores plotted against the reciprocals of the corresponding temperatures (abscissae).

Note on the Determination of Temperature Coefficients. In biological studies a usual way of estimating the effect of temperature upon a process is something as follows. When growth—to take this as an example—is studied, parallel cultures—whether of plants, fungi, bacteria or whatever is the subject under investigation—are set up usually in series at the different temperatures to be examined, and after the lapse of a certain time (an hour, a day, a week or whatever it may be) the amount of growth that has occurred is measured. If the result of such determination shows that twice as much growth has occurred at one temperature as at another, then it is said that a rise of so many degrees has doubled the rate of growth. This statement, of course, is true enough if one defines the word rate appropriately; and it would be true in general, if the growth or other process proceeded at a uniform speed throughout the life of the growing organism, or at least throughout the period of observation. But if that be not the case, and only rarely is it the case, to deduce the effect of temperature by such a method is misleading, because it is applicable only to the special time selected. If, instead of taking one day or hour, the observations were made after two days or hours, a different result would be obtained; and so after three, four or any other number of periods: each will give a different result. In short, the result obtained depends on the time arbitrarily taken as the period of observation.

An illustration from a chemical reaction will make this clear. In Fig. 5 are plotted the amounts of work done in successive units of time, when the reaction is going at two different rates, one twice that of the other—*i.e.* corresponding to a temperature difference of about 10° C. It is apparent that if one compares the amounts of work done after equal periods of time, the result will vary according to the period selected. A short time will give nearly twice as much work done by the quicker reaction, a longer time only one and a half times as much, whilst a still longer time will give almost the same amount done by both. If these were reactions running at different temperatures, one would say that the effect of the rise of temperature had been to double the rate, or to raise it only one and a half times, or even to make practically no difference, according to the time one allowed to elapse before making the comparison. One would not get a true answer at any finite time.

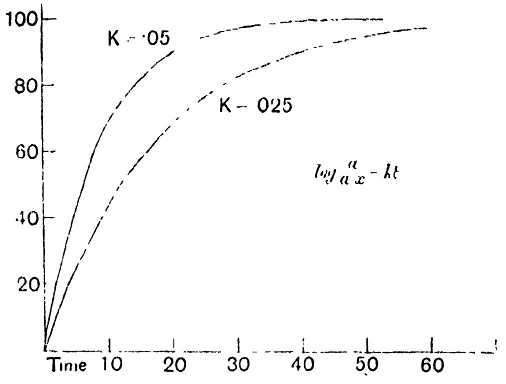

Fig. 5. Curve of unimolecular reaction proceeding at two rates, one twice that of the other.

There are two ways of looking at *rate*. One may take it as the amount of work done in a fixed time; which is the way we have been considering, or one may take it as the time required to do a certain amount of work. If the process under examination is going at uniform speed throughout, the two methods will give the same answer. The lines in the figure would be straight lines at different angles. But if it is not going at uniform speed, it is not indifferent which way is chosen, because different results are obtained. In the figure, we have seen that the first way gives variable results; but the second way gives a constant and correct result. The time required to do the same amount of work is always double what it is in the other, whatever the amount of work we select as the standard. It would seem that this must be the correct method to employ in order to arrive at a true estimate of the effect of temperature[1].

An actual example from a recent most interesting and careful study by Fawcett (9) of the growth in diameter of fungal colonies will serve to illustrate the discrepancies found in practice. Fawcett found the temperature coefficient of growth of Pythiacystis for 10° C. rises to vary according to the time of observation over a long range of temperature, from 8° C. to 31° C., and tabulates the results accordingly. Two examples, taken from his paper, are given in Table IX. If however from his data one determines the times required for the colonies to reach a certain size and calculates the coefficients from these figures, one obtains the results shown in the second half of the table, viz. consistent values. The method of calculating from the results reached in constant time gives values which vary with the time chosen; whereas the other method of calculating from the times taken to reach a constant result gives values which are consistent and independent of the standard chosen.

Table IX.

Temp. Range	Coeff. calculated from amount of growth in			Coeff. calculated from time required for a growth of	
	1st day	2nd day	4th day	12·5 mm.	25 mm.
12°-22°	4·4	3·2	2·9	2·58	2·65
18°-28°	2·2	1·5	1·2	1·60	1·50

Again, in Lehenbauer's frequently quoted paper on the growth of maize seedlings (10), the temperature coefficients are calculated from the sizes reached after 12 hours' growth, at different temperatures from 12° C.-43° C.; and, as the writer himself points out, are valid only for the time period selected. From the data given the times required to reach definite limits of size can be ascertained, and from these times the coefficients have been calculated for the sizes of 1 mm., 4·8 mm. and 7 mm. (the data do not cover greater sizes except for a few temperatures). The results differ considerably from the coefficients obtained by Lehenbauer's method, but are reasonably consistent with one another as may be seen from the examples given in Table X.

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[1] While this paper was in the press, it was found that this method had been pointed out and discussed by Osterhout in 1918 (11). Cf. also Osterhout's monograph (12).

It is not, of course, to be expected that even by the second method consistent results shall be obtained throughout the whole range of temperatures and the whole range of growth. This could only happen if the growth at all temperatures proceeded in similar fashion but at different speeds; and, as one knows, this is not the case: the shape of the growth curve alters markedly at high temperatures, especially above the optimum. This is a fact which affects all methods of calculating coefficients in such processes as growth. Even so, however, the influence of temperature on the rate of the process is more accurately represented by the second method than by that commonly adopted in biological literature.

Table X.

Temp. range	Coeff. calculated from growth reached in 12 hrs.	Coeff. calculated from times required to grow		
		1 mm.	4·8 mm.	7 mm.
14°–24°	4·17	3·1	3·1	3·1
23°–33°	1·57	1·36	1·42	1·45

I have pleasure in thanking Mr R. A. Fisher and the Statistical department at Rothamsted for assistance and advice in the application of the frequency curve used in this paper.

SUMMARY.

The spores of *Botrytis cinerea* were exposed to the action of hot water at temperatures ranging from 31° C. to 50·3° C., the proportion of spores surviving at different times determined, and the results plotted for each temperature. These give a series of approximately symmetrical sigmoid curves, all exactly alike one another except for the change in the speed of killing at different temperatures. The curves are superimposable on adjustment of the time scale, and in this respect they differ from the curves obtained with phenol, where the shape changes from an S to a J shape as the strength of phenol is increased, eventually becoming logarithmic.

The shape of the general curve agrees excellently with a recognised type of frequency distribution, and the observations at all the temperatures examined fall closely on the curve of this distribution.

The effect of temperature on the velocity of the reaction is unusually great, and is well expressed by the formula of Arrhenius, if the temperature is reckoned from 0° C. instead of the absolute temperature.

By combination of the formula for the curve and the formula for the velocity-temperature relationship it is possible to express completely for the spores of *Botrytis* the whole of the killing process for any temperature within the limits studied under the conditions and with the strain used in these experiments.

It is pointed out that in the estimation of temperature coefficients comparison of the times taken to reach a constant result give more accurate and consistent values than comparison of the results reached in constant time.

REFERENCES.

(1) SMITH, J. HENDERSON (1921). *Ann. Applied Biol.* VIII, p. 27.
(2) WEISS, H. (1921). *Journ. Infect. Diseases,* 28, p. 70.
(3) STUDENT (1908). *Biometrika,* VI, p. 1.
(4) *Tables for Statisticians and Biometricians,* ed. K. Pearson, 1914. Cambridge.
(5) MADSEN and STRENG (1909). *Ztschr. f. physikal. Chemie.* LXX, p. 263.
(6) FAMULENER and MADSEN (1909). *Biochem. Ztschr.* XI, p. 186.
(7) CHICK and MARTIN (1910). *Journ. Physiol.* XL, 404.
(8) CHICK, H. (1908). *Journ. of Hyg.* VIII, p. 92.
(9) FAWCETT, H. S. (1921). *Univ. of California Publ. in Agric. Sci.* IV, p. 183.
(10) LEHENBAUER, P. (1914). *Physiol. Res.* I, p. 247.
(11) OSTERHOUT, W. J. V. (1918). *Science,* N.S. XLV, p. 172.
(12) OSTERHOUT, W. J. V. (1923). *Injury, Recovery and Death in relation to Conductivity and Permeability.* (Monographs on Experimental Biology.)

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OBSERVATIONS ON THE BIONOMICS OF THE APPLE-BLOSSOM WEEVIL, *ANTHONOMUS POMORUM*, LINN.[1]

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(*From the Agricultural and Horticultural Research Station, Long Ashton, Bristol.*)

(With Plates XIX and XX and 11 Text-figures.)

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1. INTRODUCTION.

YEAR by year much damage is caused by the larvae of *Anthonomus pomorum*, Linn., the Apple-Blossom Weevil, and enquiries as to preventive and remedial measures are constantly received at the Advisory Centres.

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[1] A grant in aid of publication has been received for this communication.

In view of this, and the fact that the life history and habits are, apparently, assumed rather than clearly understood, it was thought advisable to spend a considerable time on the careful observation and recording of the facts of the life history, habits and limiting environmental factors, if any, of this weevil.

Thus after some discussion of the matter with Mr A. Roebuck, Adviser in Entomology, Harper-Adams Agricultural College, and Mr A. H. Lees, M.A., Entomologist and Plant Pathologist at Long Ashton, it was decided to undertake the work. Accordingly, in September 1920, an excursion into the Pershore district of Worcestershire was arranged and the weevils hunted down in some of their winter quarters and a supply secured for laboratory work during the winter.

The laboratory work, through the kindness of Professor B. T. P. Barker, M.A., was carried out at the Agricultural and Horticultural Research Station, Long Ashton, under the supervision of Mr A. H. Lees whose constant advice and suggestions have been invaluable; the outside observations have been made, for the most part, in the plantations of the Research Station supplemented by those collected in Worcestershire and in Gloucestershire.

2. METHODS AND MATERIAL.

The scheme of work was arranged as follows:

September, 1920. Inspection of plantations to discover traces of, or winter quarters of, the weevil.

October, 1920 *to March*, 1921. Weevils kept under observation in the laboratory where numerous specimens were dissected with a view to becoming conversant with their anatomy and physiology and to distinguish the sexes. At the same time preliminary spraying trials were conducted from which the most promising were selected for plantation trials. By keeping the adults indoors in the warmer conditions and by close observation of their behaviour in spring, it was possible to follow, and to anticipate, to some extent, their activities in the plantations.

March, 1921 *to May*, 1921. Outside observation of habits. Indoor control of life history.

May, 1921 *to June*, 1921. Observation in plantations of habits of young adults.

July, 1921 *to September*, 1921. Observation in plantations of feeding habits and choice of winter quarters.

September, 1921 *to Spring*, 1922. Spraying trials[1] at Long Ashton and in Worcestershire.

Spring, 1922 *to early Summer*, 1922. Verification of habits and life history and collection of information as to the efficiency of the sprays used.

3. LOCALITY AND DISTRIBUTION.

Since local conditions vary to such a great extent and with them, more or less, the habits of any particular insect, it will perhaps be helpful to say a few words about this particular district in connexion with the climate, altitude, and soil. The plantations at Long Ashton are situated for the most part in the valley; throughout the village, and the district generally, numbers of old orchards are to be found, also large numbers of isolated trees of apple, pear and plum. The area is well wooded, elms predominating.

During the winter of 1920, as stated elsewhere (2), several old cider apple trees in land adjoining the plantations were cut down, and examination of the bark of these trees revealed the presence of large numbers of hibernating weevils.

There is little doubt that with the shelter afforded by old trees in close proximity to plantations, infestation is likely to occur year after year. As well as considering the plantations, therefore, it is necessary to take these old trees into account when arranging the annual programme of spraying operations.

Since the weevil is most active in bright sunshine the amount of sunshine in any particular district will influence, to some extent, the severity of attack.

With regard to distribution *Anthonomus pomorum* is recorded as generally distributed over the British Isles, but according to Collinge (3) it occurs in reduced numbers in Scotland. In Europe it is reported from France, Germany, Holland, Austria, Russia, Norway, Sweden, Denmark, Spain, Italy, Switzerland and Trans-Caucasia. It has also been recorded in the United States.

The weevil has been known as a pest for a considerable period, Knight (4) recording it over a century ago as a serious pest.

4. LIFE HISTORY AS OBSERVED AT LONG ASHTON.

In the spring when the buds are in the "delayed dormant" stage the weevil leaves its winter quarters and, crawling up the trees, proceeds to feed by piercing the opening buds and sucking the juices. This feeding goes on principally in bright sunshine, more weevils being found feeding at mid-day than at any other time.

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[1] Recorded in *Journal of Pomology* (Vol. III. Nov. 1922, p. 54).

After feeding for a few hours the insects retire to some shelter such as bark crevices and branch crotches and remain quiescent for a time. In this manner alternate feeding and resting go on for a period of about fourteen to eighteen days during which time copulation takes place. The only references to this spring feeding that have been found are one in *Pflanzenkrankheiten* by Sorauer, Vol. III, p. 535, and another in the *Zeitschrift für Pflanzenkrankheiten*, where a paper by Schulz (5) is abstracted and the following stated, "Ende März bezw. fliegen die Käfer in die Kronen von Apfelbäumen, müssen hier aber noch etwa 14 Tage den Inhalt von Knospen ausfressen, bevor die geschlechtsreif werden." By the end of this period the apple trees are in the "cluster-bud" stage and oviposition takes place, the eggs being laid inside the flower buds. After eight to thirteen days the larva hatches and proceeds to feed on the stamens and styles of the unopened flower bud. The infested bud does not open in the normal way but the petals remain closed and dome-shaped; they turn brown and dry, thus forming shelter and protection to the delicate larva (Fig. 1). These infested blossoms are known as "capped blossoms" and possess a very characteristic appearance; they begin to be noticed by the end of April. The larva feeds for about three weeks and when fully fed, usually by the end of the first fortnight in May, pupates. The pupal stage lasts for five to ten days according to the weather, at the end of which period the pupal coat is shed and the imago appears. The perfect insect remains inside the "capped blossom" for a day or two while its exo-skeleton is hardening up, and then cuts its way out. An exit hole is seen at *A* (Fig. C, Pl. XIX). Until the end of June or beginning of

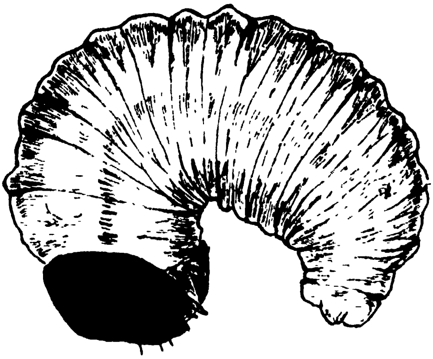

Fig. 1. 1st instar larva of *Anthonomus pomorum*. Approx. × 50.

July these young adults feed on the underside of the leaves of the apple and plum, the damage they do greatly resembling that done by one species of Tortrix moth[1]. Fig. D, Pl. XX, shows specimens of leaves upon which the weevils have fed.

Some authors maintain that the young adults hibernate without having fed. This is erroneous as shown above and, in addition, by an experiment carried out at Long Ashton in 1921, when about eighty capped blossoms were collected and the weevils on emerging were kept without food. These all died within about a fortnight after emergence.

By June 16th the young adults commenced seeking their winter quarters, a spell of cold weather about June 27th expediting matters.

The life cycle of *Anthonomus pomorum* may be summarised as follows

Year	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
1920	- -	—	- -	—	- -	—	- -	—	AW	AW	AW	AW
1921	AW	AW	AA	AA, E, L	L, P, AA	AA	AW	AW	-	—	—	- -

AW = Adult in winter quarters. AA = Adult active. E = Egg. L = Larva. P = Pupa

A. THE OVERWINTERING ADULT.

From September onwards through the winter the weevils were taken in a variety of winter quarters. In Worcestershire they were found under rough bark of plum trees, in curled up leaves both on the tree and on the ground, and in the surface soil; the most fruitful results, however, as shown by Lees (1), were yielded by the examination of the old grease bands. Under these the weevils were found in considerable numbers, for the most part, from 3 to 15 in close proximity to each other.

The grease bands were on both plum and apple trees and afforded practically the only available shelter, for the apples, especially, were very smooth barked; the plums, however, had split and creviced bark which would account for the weevils seeking them. Some few were found in the grease, having been caught while seeking winter quarters.

Later, in Gloucestershire, numbers were found under the bark of dessert pears and in the bark crevices of the younger orchard trees. In mid-winter specimens were found under the bark of isolated crab apple trees quite a mile distant from the nearest orchard; odd specimens were taken on elms and oaks near orchards.

At Long Ashton the principal winter quarters were rough bark of old cider trees, curled leaves, and under paper of grease bands. Subsequently numbers were found in the bark of the large branches from the fork upwards. The noticeable feature in reference to overwintering on the trees was that the weevils selected a position where there was pressure exerted on them from above; in no instance were they taken under the dry loose bark, their favourite places being not too dry and not too moist but always possessing this characteristic of pressure from above.

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[1] This particular species fastens a leaf down with silken strands on to an apple and feeds, under this cover, on the under surface of the leaf.

Certain other of the *Rhyncophora* were frequently associated with *Anthonomus* in its winter quarters. These included:

Anthonomus ulmi, De G.
Apion sp.
Cionus scrophulariae, L.
Cossonus ferrugineus, Clairv.
Hypera nigrirostris.
Mecinus pyraster, Herbst.
Orchestes sp.
Rhyncites coeruleus, De G.
Sitones lineatus, L.

(i) *Description of the Adult.*

The description given by Fowler(6) allows the weevil to be readily recognised. He describes it as follows:

> "Pitchy black or fuscous black, clothed with ashy pubescence; head thickly pubescent, with an impression on forehead; rostrum long and rather slender, slightly curved, antennae long and slender, reddish fuscous at apex; thorax pitchy, sometimes rugo-piceous at sides, not much longer than broad, closely punctured, with rather scanty coarse white pubescence; scutellum thickly pubescent; elytra pitchy, sometimes pitchy ferruginous, with alternate denuded and white fasciae, the one behind middle, composed of whitish pubescence, being oblique and much the most conspicuous, punctured striae distinct and rather strong, interstices very closely punctured; legs more or less pitchy, but variable in colour, femora always more or less dusky, anterior pair with a very strong tooth, intermediate and posterior pairs with much smaller teeth. L. 3 4 mm."

(ii) *Sexual Differentiation in Anthonomus pomorum.*

The most reliable sex differentiating characters as given by Fowler are as follows:

> "The males may be distinguished from the females by having the rostrum proportionately a little shorter, and, as a rule, duller, and the last ventral segment is sometimes somewhat variable in the sexes."

Owing to the great range of variation no dependence can be put on the proportionate size of male and female for many very small specimens, on dissection, proved to be females and numerous large ones males. The best distinguishing feature is the rostrum. At first it is by no means easy to decide from the rostrum the sex of a given specimen, but when the character is once definitely fixed and decided upon it becomes, with practice, quite an easy matter. The rostrum of the female is slenderly spatulate, is darker than that of the male and has a more polished appearance.

The two specimens figured, Fig. E, Pl. XX, show the following differences in measurement of length of rostrum:

Rostrum 1·25 mm. Total length 5·5 mm.
„ 0·75 mm. „ „ 4·5 mm.

Mouth parts. These are typical of the *Rhyncophora* and call for no special discussion, the accompanying figure (Fig. 2) being self-explanatory.

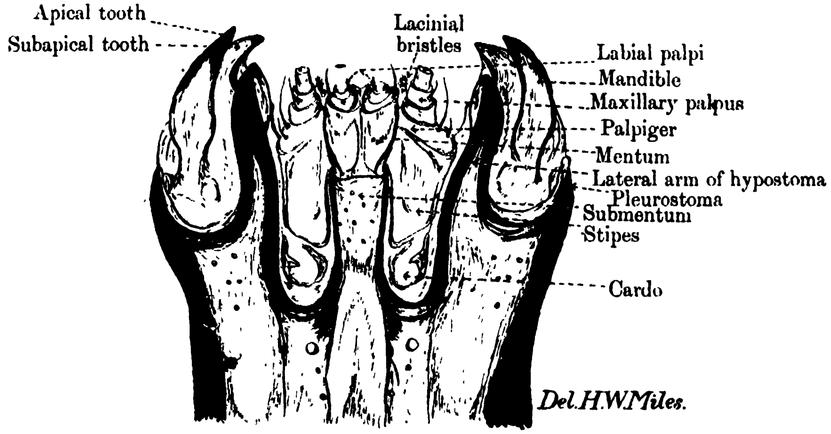

Fig. 2. Ventral aspect of mouth parts of *Anthonomus pomorum*. Highly magnified.

(iii) *Internal structure of Anthonomus pomorum*, Linn.

The Fat-body. (*Corpus adiposum.*) In *Anthonomus* this consists of a mass of tissue which almost completely envelopes the alimentary system. There is a ground tissue which contains large fat cells, and interlacing throughout the mass are fine tracheal fibrils. The colour varies from pale yellow, in the newly emerged adult, to a very rich yellow in the fully fed, and overwintering adult. The function of the fat-body is, apparently, fat storage. Obscure physiological processes are said to take place in the fat-body, some authorities connecting it with respiration, others with nutrition, while Marchal (7) associates it with the accumulation of urates.

The Alimentary System. The three primary regions are well differentiated; these are stomodaeum, consisting of mouth, pharynx, oesophagus and proventriculus; mesenteron, consisting of the ventriculus with the caecal glands; and proctodaeum consisting of the intestines with Malpighian tubes, colon, rectum and anus. These regions and their divisions can all be readily located in the accompanying figure (Fig. 3). The proventriculus is an organ possessing a highly developed chitinous structure which adapts it for masticating purposes during that period when the insect feeds on leaf tissue. It consists of eight double rows of chitinous plates clothed with innumerable denticular ridges and associated with a system of circular muscles (Fig. 4). Packard (8) referring to the function of the proventriculus states, "It has been compared with the gizzard of birds and is usually called by the German authors the chewing or masticating stomach (Kaumagen)." The posterior portion of the proventriculus projects for some little distance into the ventriculus. The mid-gut is divided into two distinct regions; the anterior is the ventriculus, a large receptacle for the macerated food which is lined with high columnar epithelial cells, and posteriorly is a constricted portion studded with caecal glands. These are short glandular bodies with large cells.

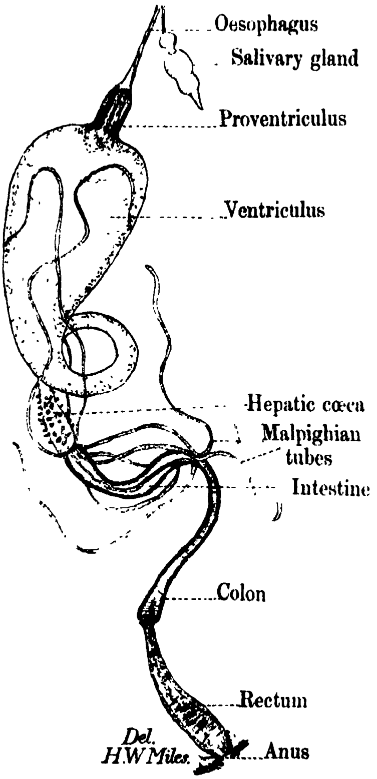

Fig. 3. Alimentary canal, *Anthonomus pomorum*. × 47½.

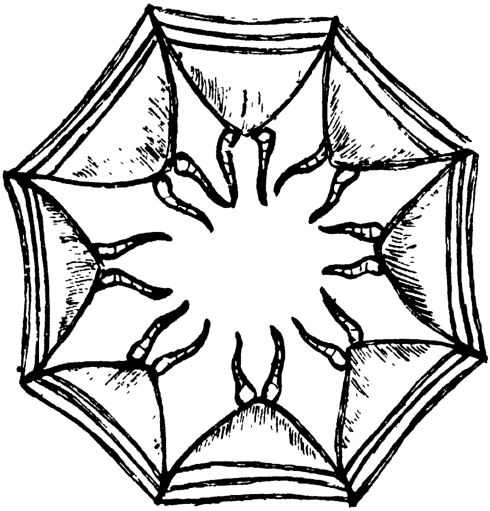

Fig. 4. Transverse section of Proventriculus, *Anthonomus pomorum*. Approx. × 400.

They are often very difficult to locate owing to their being intimately associated with parts of the fat-body and liable to come away with it on dissection.

At the junction of the mesenteron and the proctodaeum the Malpighian tubes enter; they are long, and much twisted, some running forwards and ramifying amongst the fat-body and around the ventriculus, and others running backwards and seeming to be connected by strands to the point where the colon runs into the rectum. The intestine is short and has slight circular and longitudinal muscles; the colon is glandular or absorbent, and the rectum is characterised by systems of well-developed circular and longitudinal muscles.

(iv) *The Reproductive Organs of Anthonomus pomorum.*

Examination of young adults soon after emergence shows the reproductive organs in an immature state, the testes in the male and the egg tubes in the female being quite undeveloped.

By the time the weevils seek their winter quarters the testes have developed and attained their normal size, but the ovaries are still undeveloped. The first noticeable change is apparent by early March, when the ovarian tubes show signs of development and by the time oviposition commences they are mature, as figured (Fig. 6).

The male reproductive organs, as figured (Fig. 5), consist of testes, *vasa deferentia*, *vesiculae seminalis*, common duct, or unpaired *vas deferens*, and penis with internal sac.

The testes are large yellowish bodies each divided into two main lobes, which in turn are again sub-divided. Each testis is covered by a thin membrane which carries the tracheal fibrils and connects the mass with the strands of the fat-body. From the testes the paired *vasa deferentia* lead into the common duct to the penis.

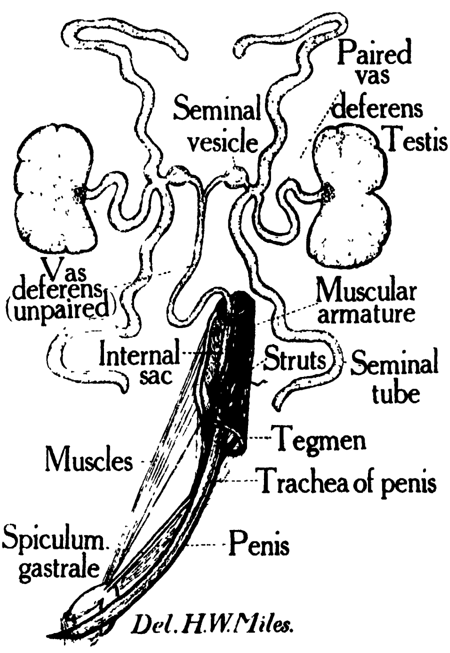

Fig. 5. Reproductive organs of male, *Anthonomus pomorum.* × 112.

Opening into the *vasa deferentia* are two seminal tubes and located immediately below their point of entry are the seminal vesicles. The unpaired *vas deferens* is a long coiled tube which leads, posteriorly, into the internal sac. This latter is located within the chitinous and muscular armature of the penis which consists of a sheath prolonged anteriorly into two struts, a tegmen or ring with a tegminal strut, and a *spiculum gastrale*.

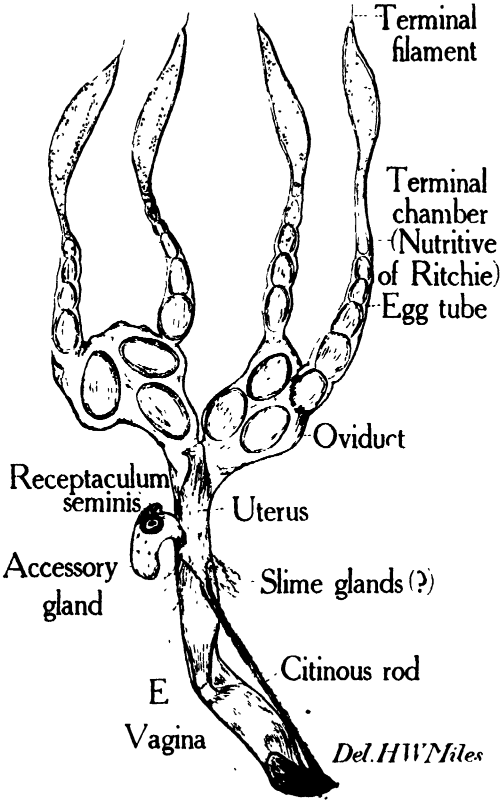

Fig. 6. Reproductive organs of mature female of *Anthonomus pomorum*. × 120.

The female reproductive organs consist of two ovaries, one on each side of the body. Each ovary is composed of two ovarian tubes opening into a common oviduct, which leads into the uterus.

At the anterior end of each egg tube a terminal filament is located; this serves to suspend or connect the ovaries with the strands of the fat-body.

The apex of each ovarian tube consists of a nutritive chamber containing cells which furnish the eggs with nutritive material. In Fig. 6 the nutritive chamber is seen to become narrower towards the point where the eggs are being formed. In the oviducts the eggs are seen to be practically fully formed. The oviducts, at their posterior extremities, lead into the common chamber, the uterus, and its lower portion, the vagina. The *bursa copulatrix* runs into the uterus; it is connected with the *receptaculum seminis* and the accessory gland. Just below the junction of the *bursa copulatrix* with the uterus, a number of fine tubules enter the uterus; their function is unknown, but it is suggested that they may be analogous to the slime glands of many *Scolytidae.* At the posterior extremity of the vagina are two heart-shaped chitinous plates produced posteriorly into small rounded papillae. A chitinous rod runs from the median region of the uterus to the posterior extremity of the vagina. The function of the chitinous plates is suggested to be the guidance of the ovum at oviposition. At this latter operation the whole of the portion marked *E* (Fig. 6) is everted.

B. Feeding Habits and Oviposition.

In order to form some idea as to the feeding habits of *Anthonomus* on emergence from winter quarters specimens were taken into the laboratory and placed on twigs of apple which had been forced into growth in the greenhouse, the buds just showing green, a stage known to the grower as the "delayed dormant," or "bud-green." The weevils put on these twigs immediately crawled upwards to a terminal bud (Fig. B, Pl. XIX) where, tearing off and rejecting the surface tissue they forced the rostrum deep into the bud and, resting practically motionless, sucked the juices. From the punctures thus made drops of sap exuded. Later the expanded leaves showed evidence of the weevil having fed, in the form of circular holes. Where these marks occur in plantations they indicate the presence of the weevil at the bud-green stage. After feeding, the weevils went into hiding in cotton-wool and in crevices between the branches. In the same way weevils were found to feed on pear buds.

It was impossible to obtain in the plantations shoots of apple in the cluster-bud stage so early, but the foregoing data were sufficient to indicate that the weevils leave their winter quarters, feed for a shorter or longer period and return to shelter.

In 1921 the first appearance of weevils outside in the plantation was on March 24th when, about mid-day and in bright sunshine, numbers were found crawling actively about the apple trees, piercing the opening buds in the process of feeding. Many were seen in copula. Feeding and resting took place until about April 6th, when ovipositing commenced, that is, about a fortnight after the first feeding and mating. The act of oviposition, as far as could be observed both in the plantations and in the laboratory under the binocular microscope, was as follows: First the pubescent outer tissue was torn off in small fragments and rejected. This was followed by a semi-rotatory and tugging movement with the feet firmly planted on the bud. The rostrum was then thrust downwards and the head rotated from side to side. The insect remained quiescent for a period varying up to 15 minutes, during which time, as revealed by subsequent examination, a cavity had been eaten in the anther lobe immediately below. The rostrum was then withdrawn and the female, reversing her position, after careful orientation, extended her ovipositor and thrust it down into the prepared aperture. In this position the female would remain for a short period, rarely longer than one minute, after which she withdrew the ovipositor and crawled either to the next bud where a similar procedure was adopted, or down into the cluster of bud-stalks to rest in a crotch. Theobald (9), referring to the act of oviposition, states that after the egg has been laid the female "turns round and pushes it far into the cavity with her snout....The hole is afterwards closed up...with saliva." In all the cases observed at Long Ashton the female was not observed to turn round and push the egg farther down into the cavity after having oviposited, and dissection of the buds selected for oviposition showed that the eggs were actually fixed into the tissue of the anther lobes in the cavities which had been eaten out for their reception (Fig. 7). As regards the closing of the puncture, though Curtis (10) also refers to such an action on the part of the female, in no instance was it observed by the writer

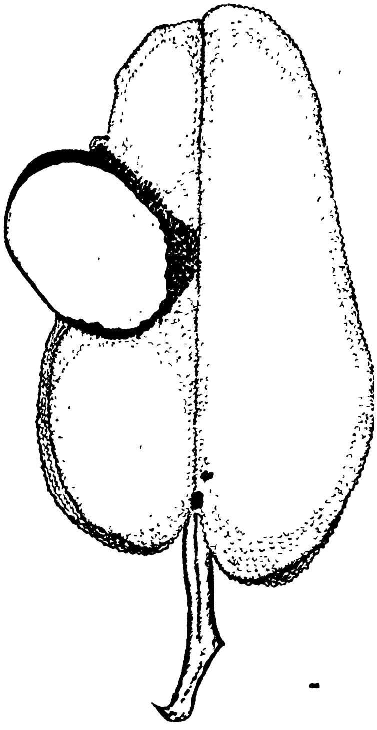

Fig. 7. Egg of *Anthonomus pomorum*, *in situ* in anther lobes of apple. ×60.

in 1921 or in 1922, but it was noticed that a sappy exudate from the pierced tissue readily filled the puncture and congealed, quite sealing up the entrance. The whole act of oviposition occupies from eighteen to twenty-five minutes[1] and in no instance was it observed to occupy three-quarters of an hour as stated by Curtis and Theobald.

While ovipositing was in progress many weevils were to be found on the trees resting in small crotches near blossom buds which showed signs, in the form of discoloured punctures, of their having fed. Some of these weevils, males and apparently unfertilised females, were collected and kept under observation in the laboratory, one specimen being observed to feed in a single puncture for upwards of two hours.

Feeding goes on for two or three weeks after the first sign of ovipositing and, towards the end of the period, the adults may be seen clinging lethargically to the buds or in small crotches on the trees; many of these were spent or unfertilised females.

One important point noticed was, that during the first bud-feeding stage and the early stages of oviposition the weevils would fold their limbs and drop from the buds at the least touch or jar, but about half-way through the ovipositing period no amount of jarring would induce them to fall.

C. THE EGG STAGE.

The eggs are broadly ovoid in shape, are densely white in colour and measure on an average ·7 mm. × ·5 mm. Where ovipositing took place in 1921 on April 5th the larvae hatched on April 18th, thus giving a period of 13 days for the duration of the egg stage. In 1922, however, eggs laid on April 28th hatched on May 6th, giving only eight days for the egg stage.

D. THE LARVAL STAGE.

On hatching, the larva is a soft white legless grub with the head comparatively large and black. The body is wrinkled into numerous folds and sparsely covered with hairs or bristles (Fig. 1). The spiracles are very minute and inconspicuous. No eye spots are present. The antennae are minute and rudimentary. Examination of numerous infested apple blossoms showed that the larvae commence feeding on the anther lobes; from these their depredations extend to the filaments and styles. Usually only one larva occurs in each capped blossom, but in 1921 in several instances two were found. The petals are gnawed partly through

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[1] In many cases, under especially favourable conditions, it only occupied twelve minutes.

at their base, with the result that though they are attached to the receptacle they do nŏt develop further but remain as a dome-shaped cap which turns brown and wrinkled as time goes on. With several moults, the larva continues feeding for fifteen to seventeen days, at the end of which period there remains nothing of the stamens or styles above the receptacle, the upper surface of which also frequently shows signs of the larva having fed. The full-fed larva presents the appearance shown in Fig. 8. It consists of a slightly retractile head, three thoracic segments and nine distinct body segments and bears numerous bristles. In the head can be discerned the labrum and clypeus on the sides of which are the mandibles. These are strongly chitinous and toothed, and below them lie the tri-segmented maxillary palpi. Posteriorly to the clypeus the epistomal and frontal areas can be seen. The frons is a triangular plate bearing several frontal bristles and behind it, occupying the greater portion of the dorsal area of the head, and bounded anteriorly by the frontal sutures, is the epicranium. This is divided into two portions by the epicranial suture, and bears several bristles (Fig. 9).

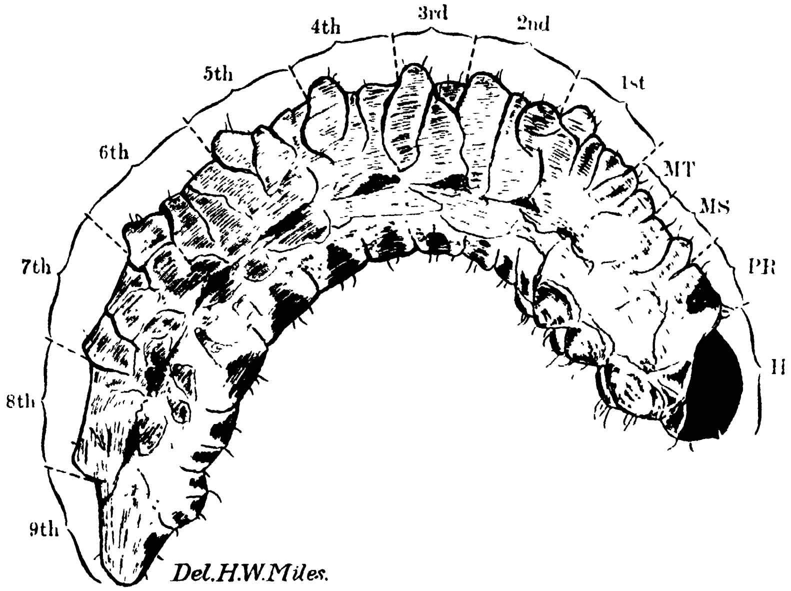


Fig. 8. Larva, *Anthonomus pomorum*. Lateral view. Approx. × 24. 1st–9th = abdominal segments. H = head, PR = pro-thorax, MS = meso-, MT = meta-thorax.

The thorax, consisting of pro-, meso-, and meta-thoracic segments, is readily located. The pro-thorax bears, dorsally, two darker, apparently

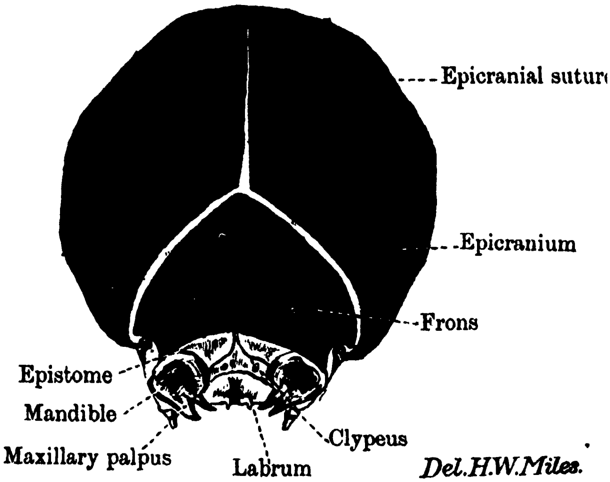
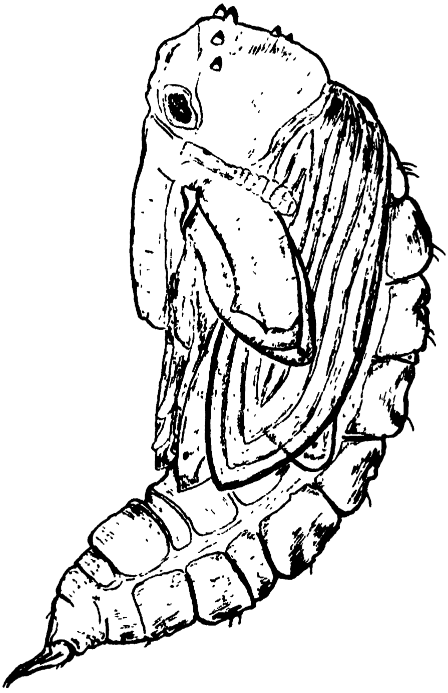


Fig 9. Head of larva, *Anthonomus pomorum*. Front view, highly magnified.



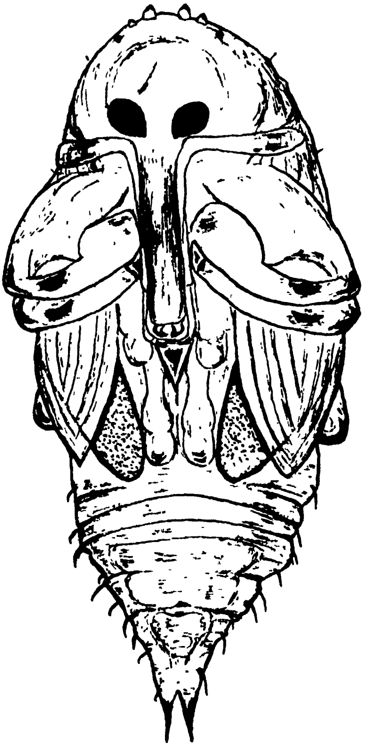


Fig. 10. Lateral view, Pupa *Anthonomus pomorum*. × 30.
Fig. 11. Pupa *Anthonomus pomorum* darkening up prior to emergence. Ventral view × 30.

chitinous areas. The other thoracic segments are divided into prescutal and scutellar lobes. The abdominal tergites show division into prescutal, scutal and scutellar lobes, of which the last named are very prominent. The divisions between the lobes are deeply cleft, which characteristic, in the sternites and pleurites, renders difficult the location of the spiracles. There are nine spiracles on each side, the first being located in the prothoracic segment and the remainder in the first to the eighth abdominal segments.

The Change from Larva to Pupa.

The fully-fed larva, which measures about 6 mm. in length, is superficially dirty white or slightly creamy in colour. Through the outer skin the brownish food contents are visible, with the result that the larva may present a very mottled appearance. At the cessation of feeding, processes go on internally whereby digestion is finished, and the indigestible residue defaecated. The larva which is now a clean light yellow colour, remains quiescent and, after about two days, pupation takes place and the larval skin is shed.

E THE PUPAL STAGE.

The general characters of the pupa are shown in Figs. 10 and 11. The pupa is soft, fleshy, and clothed laterally and dorsally with numerous stout bristles. The legs, elytra, wings, antennae and rostrum are folded in characteristic fashion, and the whole is swathed in a thin transparent pupal coat. The pro-thorax bears numerous sharp conical tubercles; it is suggested that these may be used to break the pupal coat at emergence. The ninth abdominal segment bears two stout caudal spines which are used to effect any movement. The length of the pupal stage is from five to eight days, or even longer.

Development and Emergence.

The first sign of development in the pupa is the darkening of the eyes which occurs, faintly, after about two days. By the third day the eyes are distinctly noticeable, the mandibles are brownish, the rostrum denser and the membranous wings are smoky. Immediately before emergence of the weevil the eyes, rostrum, and antennae are dark and the dorsal area of the pro-thorax and the margins of the meso- and metathoracic tergites are also denser. The tarsal claws are next to darken, followed by the articulations of the limbs, and the tip of the abdomen: still later the femora darken and the femoral teeth become very distinct.

At emergence the pupal coat splits along the line of pro-thoracic tubercles and forward down the rostrum. The clubs of the antennae are forced apart, the forelegs are slightly extended, and periodic abdominal movements may be seen. These rotary abdominal movements result in the pupal coat being forced backwards. The clubs of the antennae and the tip of the rostrum remain attached to the pupal coat for some short time; finally, however, they are freed and only the posterior tarsi retain the coat until the caudal portion comes away. The whole process takes about three-quarters of an hour. After emergence the legs are stretched and moved, the membranous wings extend to their full length and are then folded, the elytra falling into place above them. The abdomen shortens until the pygidium is drawn beneath the elytra. The chitinous structures harden up rapidly and the elytra assume their dark colour above and below the "V" mark.

F. THE YOUNG ADULT.

On emergence from the capped blossoms the young adults of *Anthonomus* are very active and will fly slightly more readily than do the overwintered adults. They feed on the petals of opened blossom and on the under surfaces of the leaves. In the latter case the upper epidermis is left intact. In captivity both apple and plum foliage were used as food.

Dissection of a male specimen three days after emergence showed the ventriculus full of macerated food and all the intestinal portion much distended. The testes were very small and undeveloped. The chitinous exo-skeleton and the elytra were soft and fragile as distinct from the tough coats of the overwintered adult. During the period at the end of May and early June various substances were used in an endeavour to find out if the young adults could be attracted for control purposes, but no success was obtained. As in the spring feeding of the overwintered adult so in the summer feeding of the newly emerged adult, it was noticeable that alternate periods of feeding and resting occurred, the weevils seeking any available cover after feeding, some re-entering the capped blossom, others crawling into crotches or bark crevices or into the herbage beneath the trees, where cover-cropping was followed.

The first emergences of new weevils in captivity were noticed on May 13th; by June 27th the fat-bodies were well developed and yellow, and the testes practically full size and yellow. On June 25th a specimen, taken from under the bark out of doors, was dissected; the stomach was empty, the fat-body was well developed but pale and the testes were practically of full size and yellowing.

From this evidence it can be concluded that the young adults, in an average season, will be fully fed by the end of June or beginning of July and from this period onwards will be seeking winter quarters. The earliest date of finding weevils in their winter quarters, under paper bands tied on the trunks of trees, was June 16th. In order to obtain some data as to the movements of the weevils towards winter quarters, three bands were placed on trees on June 10th. These were examined with the result that weevils were collected as follows:

June, 34; July, 25; August, 7; September, 12; October, 5.

This indicates that the majority of weevils seek winter quarters in June and July.

5. FACTORS LIMITING THE DISTRIBUTION AND EXTENT OF *ANTHONOMUS POMORUM*.

The chief factors limiting the distribution of *Anthonomus pomorum* are:

A. The distribution and extent of its host trees which are: (*a*) apple, (*b*) pear, (*c*) crab apple.

B. Climatic conditions.

C. Natural enemies: birds, insects and fungi.

A. DISTRIBUTION AND EXTENT OF HOST TREES.

In the west of England apples and pears are cultivated extensively in an area consisting of Somerset, Gloucester, Hereford, Worcester and parts of Monmouth, Dorset, Oxford and Wiltshire, and throughout this area the conditions are such as to favour the persistence of the weevil, viz. a mixed tenure composed of intermingling old cider orchards, and plantations of younger trees of choice varieties. The old cider orchards furnish ample winter quarters, and in spring numbers of overwintered adults spread to adjoining plantations, where they oviposit. Older orchards are therefore a source from which migration, to a greater or less extent, will constantly take place. Isolated trees are also infested, and during the late winter and early spring of 1920–21 hibernating adults and "capped" blossoms were found on crab apple trees, some of which were over a mile from the nearest orchard.

B. CLIMATIC CONDITIONS.

As regards the climatic factor, the main influence is felt in spring, during the latter part of March and on to the end of April. Bright sunshine and warm days make the weevil leave its winter quarters and seek the buds for food. Following the first feeding period there is a lapse of from one to two weeks, pending the ripening of the ovaries for oviposition; and if, during this period, bright warm days continue, the development of the blossom-buds is very rapid and, by the time the female is ready to lay her eggs, a considerable number of buds may have passed the ideal stage, the cluster-bud stage, and others have just sufficiently developed as to open during the ten days or so of incubation, and the young larvae having no protection, perish, resulting in a minimum of loss through weevil attack.

On the other hand, when the period of blossom-bud development is protracted or checked by cold and dull spells of weather, the weevils have their period for ovipositing considerably lengthened, and so the loss due to capped blossom is considerably increased.

C. NATURAL ENEMIES.

(i) *Birds.*

Undoubtedly birds are of considerable importance in keeping in check the apple-blossom weevil. Though no special observations in this connexion were made at Long Ashton, from time to time several species have been reported as definitely destroying *Anthonomus* sp.; these are—woodpeckers, tits and sparrows. Theobald (9) states that woodpeckers are of use, while Collinge (11) records finding specimens of *Anthonomus pomorum* amongst the stomach contents of the blue-tit. With regard to sparrows, Maarschalk (12) gives an instance of their pecking open capped blossoms and destroying the larvae; this was in Holland, but so far no similar record has been made in England. Newstead (13) states, in reference to the marsh-tit, that it "is responsible for the capture of some specimens of the apple blossom weevil." In Gloucestershire it was particularly noticeable that, in the spring of 1921 when *Anthonomus pomorum* was egg-laying, the nut-hatch (*Sitta caesia*, Wolf.) was very active, and spent several weeks feeding in the orchards.

(ii) *Insects.*

Other natural enemies of *Anthonomus* occur among insects and include one species from the Thysanoptera, *Haplothrips tritici* (Kurd.), a predaceous thrips, which is recorded by Kurdjumov (14) as feeding, *inter alia*, on the larvae. A number of species of the order Hymenoptera are parasitic on the larva and pupa of the weevil.

Imms(15) gives a summary of the species recorded from *Anthonomus pomorum*; this includes:

1. *Pimpla pomorum*, Ratz.
2. *Pimpla examinator*, Fab.
3. *Pimpla graminellae*, Grav.
4. *Pimpla sagax*, Stg.
5. *Apanteles lacteus*, Nees.
6. *Apanteles impurus*, Nees.
7. *Campolex latus*, Ratz.
8. *Meteorus ictericus*, Nees.
9. *Habrocytus fasciatus*, Thoms.

Only one of these species was observed at Long Ashton, viz. *Pimpla pomorum*, Ratz., specimens of which were submitted to Dr Imms who forwarded them for identification to Mr Claude Morley.

The larvae of this species are ecto-parasitic in habit, feeding on the larva and pupa of the host. In his summary of "Observations on *Pimpla pomorum*," Imms states, "The larva of this Ichneumon is composed of a definite head and thirteen trunk segments; no caudal process or anal vesicle is present. Ten pairs of spiracles are evident, but the pair situated on the second segment is minute and vestigial. Pupation takes place within a slight silken cocoon spun within the cavity of the unopened apple buds." The adults emerge about twenty-three days after the cocoons are spun. Where the *Anthonomus* larva is attacked, the parasite is usually located, as shown by Imms (*op. cit.*), on its dorsal side, but in the case of the pupa the anal extremity is selected. Half-devoured pupae of *Anthonomus* are very common in the capped blossom, and Imms, referring to these, states that, since the remaining portion shows no signs of discoloration or putrefaction, "it appears not unlikely that the parasitic larva may inject some kind of secretion into the tissues of its host, serving as an antiseptic arresting decomposition."

With regard to the efficiency as a parasite, of this species, at Long Ashton the parasitism worked out at about 5 per cent., twelve specimens of *Pimpla* being reared from 238 capped blossoms.

Imms gives an instance of 27·4 per cent. parasitism in buds examined in 1916 and quotes Decaux as rearing and liberating about 250,000 parasites from the infested buds collected from 800 apple trees.

The specimens reared at Long Ashton included both males and females. The female has been known since 1848 when Ratzeberg described it, but few records of the male exist and of these the first English specimens were recorded from Cambridgeshire in 1916 by Imms for whom a full description of the sex was drawn up by Morley.

The occurrence of males in widely separated areas like Cambridgeshire and Somerset suggests a probable fairly wide distribution of this sex.

In January, 1921, an endo-parasitic larva was taken from a specimen of *Anthonomus pomorum* sent in from Worcestershire. This was submitted for examination to Dr Imms who reported on it as follows:

"Your larva is most certainly that of an Ichneumon in the first instar —the caudal prolongation is a common feature among endo-parasitic *Ichneumonidae.*"

This parasite was located in a male weevil between the testes and high up towards the dorsal region of the abdomen; the testes were very pale in colour and the fat-body was tinged with brown as distinct from its fresh yellow colour in healthy specimens.

No adults of internal parasites were reared from overwintered weevils.

(iii) *Fungi.*

In the spring of 1921 examination of some old orchard trees showed numbers of *Anthonomus* dead and heavily infected with a fungus which was apparently a species of *Isaria*. The mortality was about 4 per cent. No other fungal parasites have been observed.

6. SUMMARY.

1. The life history of the Apple-Blossom Weevil, *Anthonomus pomorum*, a serious pest in apple-growing districts, has been worked out and is herein described.

2. The adult, of which a full description, including that of the alimentary and genital systems, is given, spends the winter hibernating in or near apple orchards.

3. In spring the adults leave their winter quarters and, prior to mating, feed by piercing the fruit buds and sucking the juices.

4. Egg laying is performed when the development of the buds is in the stage known as "Cluster-bud." The egg is thrust into the centre of the young flower by the ovipositor of the female.

5. The stages, oval, larval, and pupal are described followed by a discussion of the habits of the young adult which is shown to feed on the under surfaces of the leaves.

6. Natural enemies, including birds, insects and fungi, are shown to play an important part in limiting the extent and distribution of the weevil.

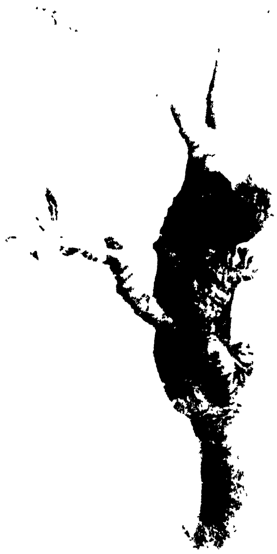


Fig. A

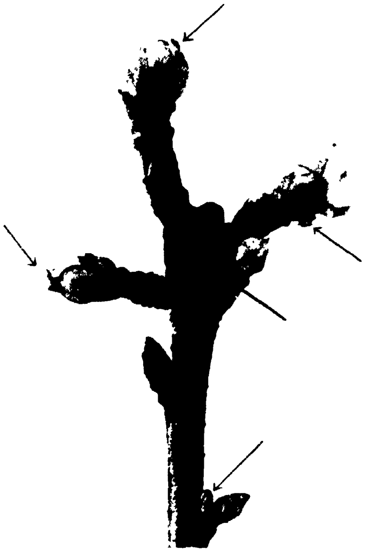


Fig. B.

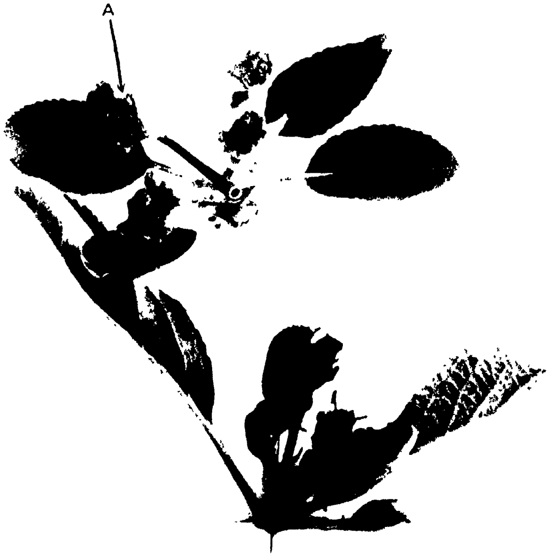


Fig. C.

MILES.—Observations on the Bionomics of the Apple-Blossom Weevil. (pp. 348—369.)

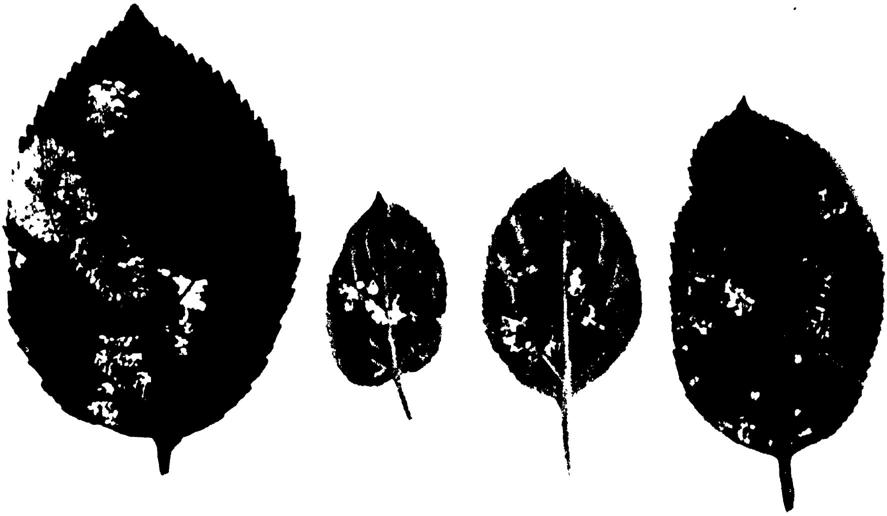


Fig. D.

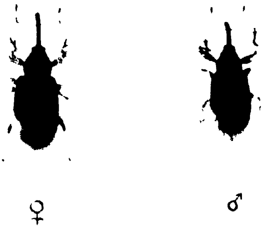


Fig. E.

MILES.—Observations on the Bionomics of the Apple-Blossom Weevil. (pp. 348 - 369.)

7. BIBLIOGRAPHY.

(1) **Lees**, A. H. (1921). *Ann. Rept. Agr. and Hort. Research Station.*

(2) **Miles**, H. W. (1921). *Journ. Pomology*, II, No. 4.

(3) **Collinge**, W. E. (1908). *Journ. Bd. of Agriculture*, xv, 674.

(4) **Knight**, T A. (1801). *Treatise on Culture Apple and Pear*, p. 98.

(5) **Schulz**, U. K. T. (1918). *Sitz. Ber. Ges. nat. Frde. Berlin*, abst. *Zeits. für Pflan.* xxx 4/5, 193.

(6) **Fowler**, W. W. (1891). *British Coleoptera.*

(7) **Marchal**. *Dictionnaire de Physiologie.* (Paris.)

(8) **Packard**, A. S. (1903). *Textbook on Entomology*, p. 306.

(9) **Theobald**, F. V. (1909). *Insect Pests of Fruit.*

(10) **Curtis**, J. (1844). *Gardeners' Chronicle*, p. 556.

(11) **Collinge**, W. E. (1913). *Food of some British Birds.* (London.)

(12) **Maarschalk**, H. (1918). *Tijdschr. Plantenzeckten, Wageningen*, xxiv, No. 4, abst. *R.A.E.A.* vi, 499.

(13) **Newstead**, R. (1908). *Supplement Journ. Bd. Agric.* xv, No. 9.

(14) **Kurdjumov**, N. V. (1913). *Trans. Poltava Agr. Expt. Stn.* No. 18, *Dept. Econ. Ent.* No. 7, abst. *R.A.E.A.* iv, 165.

(15) **Imms**, A. D. (1917). *Ann. App. Biol.* iv, No. 4.

EXPLANATION OF PLATES XIX AND XX.

Fig. A. A type of canker spot often selected by *Anthonomus* as winter quarters. (× ½.)

Fig. B. *Anthonomi* feeding on apple buds in spring. The buds are in the "delayed dormant" stage. Note weevils resting in crotches. (Nat. size.)

Fig. C. "Capped" blossom. A = exit hole made by young adults on emergence. (Nat. size.)

Fig. D. Leaves of apple damaged by the feeding of young adults of *Anthonomus pomorum*, L. (Nat. size.)

Fig. E. Adults of *Anthonomus pomorum*, showing difference in size and in length of rostrum. (× 3·8.)

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A STUDY OF A CAUSE OF "BLOWING" IN TINS OF SWEETENED CONDENSED MILK

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The work described below had its origin in the fact that a tin of sweetened condensed milk was found to be so badly "blown" that it burst on handling. In order to seek the cause of this defect, portions of the contents of the tin were inoculated into liquid media of various kinds and incubated. Gas production occurred in beer-wort and in sterilised milk to which cane sugar had been added. Microscopical examination demonstrated the presence of numerous yeast cells in those tubes which had shown fermentation. Plates were then made on beer-wort agar and yeasts of two types were found. The one gave heavy white colonies consisting of round cells (described as "*A*" in the text); the other type of colony grew more slowly, was translucent, and consisted of oval cells (described as "*B*" in the text). Both these yeasts were capable of producing gas in sterilised milk containing a high percentage of cane sugar. These facts having been ascertained, a more detailed study of each type was carried out with cultures obtained from single cells. Hansen's (3) method, using a Böttcher chamber, was adopted for the isolation of *A*, and Barber's (1) method for *B*.

MORPHOLOGY AND CULTURAL REACTIONS OF YEAST *A*.

This type consisted of round cells of diameter 5–6 μ. In old cultures a number of larger cells were found, some of which attained a diameter of 8·5 μ. Spores have not been obtained either on thin layers of gelatine or on gypsum blocks.

A streak culture of this yeast on beer-wort agar gave a heavy white growth which tended to become brownish and "granular" in old cultures. In beer-wort agar stab cultures there was growth throughout the stab and good growth at the surface. In beer-wort vigorous fermentation was set up and frequently a ring of scum was formed around the sides of the tube.

This yeast readily fermented Saccharose, Dextrose and Laevulose, but neither Lactose nor Maltose. There was a doubtful fermentation of Galactose. No fermentation was set up in milk, but when cane sugar was added, even in a concentration of 70 per cent., gas production and coagulation of the milk occurred.

In order to test whether this yeast was able to cause "blowing," growth from cultures on beer-wort agar was introduced into tins of sweetened condensed milk, which were then securely re-sealed and placed in an incubator at 22° C. At the end of ten to seventeen days the tins were slightly "blown," and one began to leak in five weeks. A similar tin which had been inoculated with a "gassy" culture in milk + 60 per cent. cane sugar showed only very slight "blowing" at the end of five weeks. Control tins of condensed milk kept under exactly the same conditions remained normal throughout the whole of these periods. When one of the blown tins was opened after three months' storage at ordinary room temperature, the contents were found to be still fluid, showing no evidence of "lumps." A considerable amount of gas had been dissolved in the milk which became "frothy" when the pressure was released. There was a slightly alcoholic taste and smell.

MORPHOLOGY AND CULTURAL REACTIONS, ETC. OF YEAST *B*.

Young cultures of this type consisted of oval cells of length 3–4·7 μ and breadth of 1·5–2·7 μ. These sometimes attained a length of 5 μ and a breadth of 3 μ in older cultures. The cells reproduced themselves by rapid budding, and since the young cells tended to hang together, small colonies were frequently found in liquid cultures. Spores have not been demonstrated either on gelatine or gypsum blocks.

A streak culture on beer-wort agar showed a light, white growth which became brown in old cultures. In stab cultures there was growth throughout the stab, but practically none at the surface. In beer-wort a vigorous fermentation was set up, but no scum was formed.

Saccharose, Dextrose and Laevulose were rapidly and vigorously fermented, but not Maltose or Lactose. A slight fermentation was produced occasionally in Galactose. In milk there was no fermentation, and very scanty growth, but in milk containing cane sugar there was rapid fermentation accompanied by coagulation of the milk. High concentrations of sugar seemed to have no inhibitory effect, for the yeast was able to cause vigorous fermentation in milk + 70 per cent. cane sugar.

When, as in the case of *A*, this yeast was inoculated into tins of sweetened condensed milk, which were then placed in an incubator at 22° C., a rapid fermentation ensued. The tins inoculated from cultures of *B* on beer-wort agar were found to be "blown" in four days and one burst in seventeen days. A tin inoculated from a "gassy" culture in milk + 60 per cent. sugar was "blown" in seventeen days. One of the "blown" tins was opened after three months' storage at ordinary room temperature. There was a strong rush of gas on piercing the lid. The contents had a distinctly etherial odour. Coagulation of the milk had taken place, and for about half the depth of the tin the milk was almost solid. The "lumps" had a stinging and slightly acid taste. The changes produced in the contents of the tins were much greater than in the case of *A*.

A study of the results published by workers in other countries suggests that defects in sweetened condensed milk having an origin similar to that described above are of frequent and wide-spread occurrence. Pethybridge (5) has demonstrated that a round and an oval yeast were the cause of "blowing" in tins of Irish condensed milk. As far as the data given allow of comparison these two types are very similar to those described ("*A*" and "*B*") although the oval appears to be somewhat smaller. Hammer (2) has isolated from American sweetened condensed milk an oval yeast which was proved to be the cause of "blowing" in the tins, and which, in its cultural reactions closely resembled *B*. It was, however, considerably smaller, and the cells showed no tendency to hang together in groups. In this connection it may be mentioned that, whereas the measurements for *B* recorded in the text were made on unstained cells, stained preparations give figures which correspond with those of Hammer. Knudsen (4) has already made this observation. Further, this yeast, *B*, rarely forms colonies when grown in milk containing a high percentage of sugar, but appears mainly as isolated cells, or cells having a bud attached. Knudsen (1) has described two types of yeast; one of these was round, and was isolated from "blown" tins of both Danish and Dutch sweetened condensed milk; the other was oval and was obtained from "gassy" Dutch sweetened condensed milk. By the courtesy of Professor Orla-Jensen the author has had the opportunity of making comparative tests with these, in conjunction with Knudsen, at the Polytechnic College, Copenhagen. There seems to be no doubt that the two types are identical with those described in this paper.

THERMAL DEATH POINT.

A study of the thermal death point of these yeasts seemed to be of considerable practical importance. Experiments were carried out on cells from cultures on beer-wort agar which had been kept at 22° C. for two days.

The cells were washed off in sterile saline, and quantities of 10 c.c. of this emulsion heated in test tubes to various temperatures, by plunging the tubes into water which was kept at a temperature 2–3° C. above that required. After heating, the tubes were plunged into cold water. Other experiments were carried out in which the tubes were kept at given temperatures for ten minutes.

To determine the vitality of the cells, measured quantities of the heated emulsions were inoculated into various media, *e.g.* beer-wort agar plates, beer-wort agar slopes, beer-wort, and milk + 60 per cent. cane sugar. The number of colonies on the plates, and the presence or absence of growth in the various media were noted.

Both *A* and *B* were able to survive an exposure to 50° C. for ten minutes while *B* was unable to resist 55° C. for ten minutes. Occasional cells of *A* were found to resist this exposure, but no growth was demonstrated after heating at 55° C. for fifteen minutes, or at 60° C. for ten minutes.

The emulsions which had been momentarily heated to given temperatures showed somewhat erratic results. The majority of the cells of both *A* and *B* were killed by momentary exposure to a temperature of 70° C. Nevertheless it has been found that occasionally cells of *A* survived a temperature of 72° C. and cells of *B* even 76° C.

It was felt, however, that it was very difficult to adjust the temperatures with any great degree of accuracy when such rapid methods of heating were adopted. A second series of experiments was carried out in which the tubes were placed in cold water and the temperature of the whole water bath gradually raised. The final temperature could thus be accurately controlled.

Second Series of Experiments. In order that the heating might be carried out under conditions which might occur in the condensed milk industry the cells were heated in milk or in cane sugar syrup. The cultures used had been grown under varying conditions for varying periods of time. Seven experiments were carried out in this series.

In the first four experiments, cultures on beer-wort agar were employed. For Exps. 1 and 3, the culture had been incubated at 22° C.

for two days, for Exp. 2, the culture had been kept at 22° C. for nine weeks, for Exp. 4 the culture had been incubated at 22° C. for two days, and kept at room temperature for nineteen weeks. In each case the cells were washed off in sterile saline, and from these emulsions measured quantities were added to tubes containing 9 c.c. of sterile 50 per cent. syrup sugar, or of sterile milk, and heated to the temperatures shown in the table. 1 c.c. of syrup was then added to each tube of milk and 1 c.c. of milk to each tube of syrup, and the tubes incubated at 22° C. The presence or absence of gas was noted.

The rate of heating of the emulsions of the organisms was kept as nearly constant as possible throughout the series. Table I gives the actual figures.

Table I.

			Time taken to raise temperature of emulsion				
Expt.	Emulsion	Initial temp.	From init. temp. to 55° C.	55–65° C.	65–73° C.	73–85° C.	85–100° C.
1 and 2	Milk	14° C.	17 mins.	12 mins.		7 mins.	9 mins.
	Syrup	14° C.	17 ,,	14 ,,		8 ,,	18 ,,
3 and 4	Milk	12° C.	18 ,,	15 ,,		8 ,,	11 ,,
	Syrup	12° C.	18 ,,	14 ,,		8 ,,	10 ,,
5 and 6	Milk	22° C.	13 ,,	6 mins.	6 mins.	10 ,,	9 ,,
	Syrup	22° C.	12 ,,	6 ,,	5 ,,	6 ,,	9 ,,
7	Syrup	21·5° C.	14 ,,	8 ,,	7 ,,	9 ,,	7 ,,

During the process of heating, the temperatures of the syrup and the milk in the inoculated tubes were taken as being the same as that of control tubes containing thermometers. The tubes were cooled by immediate plunging into cold water.

Exps. 5, 6 and 7 were carried out in a slightly different manner, since it was considered advisable to study the growth of the heated cells in milk containing a high percentage of sugar. The tests on the vitality of the cells were, therefore, made in milk to which 60 per cent. cane sugar had been added.

In Exp. 5, a culture on beer-wort agar which had been incubated at 22° C. for four days, and kept at 37° C. for seven days, was used. Emulsions were made in sterile whole milk, and sterile 50 per cent. cane sugar syrup, and quantities of 3 c.c. heated to the temperatures shown in Table I. After heating and cooling, 1 c.c. from each sample was inoculated into milk + 60 per cent. sugar and incubated at 22° C.

For Exp. 6, a culture on nutrient agar, to which 60 per cent. cane sugar had been added, was incubated at 22° C. for four days and kept at

37° C. for seven days. An emulsion was made in 50 per cent. cane sugar syrup, and 5 c.c. quantities were heated in the usual manner. From these, inoculations were made into milk + 60 per cent. sugar, and the tubes incubated at 22° C.

Exp. 7 was devised to investigate whether these yeasts were likely to remain alive for a prolonged period, or to develop an increased resistance to heat, when stored on sugar. Cane sugar was placed in flasks and sterilised by autoclaving, so that it formed a very thick syrup containing numerous sugar crystals. As much as possible of the supernatant liquid was removed from a vigorous culture of each yeast in beer-wort, and the residues, consisting mainly of yeast cells, placed on this sterilised sugar, and kept at 30° C. for three weeks and then at room temperature for seven weeks. The cells were then washed off with a sterile sugar syrup and heated as in previous experiments. Inoculations were made into milk + 60 per cent. sugar and the tubes incubated at 22° C.

In every case except Exp. 5, where the fact that the emulsion had been made in milk rendered it impossible, the number of cells introduced into each tube was determined by the Haemocytometer method.

RESULTS.

The results obtained are shown in Table II which demonstrates:

1. That under the conditions of these experiments *B* was killed at a temperature between 55° and 65° C., whilst *A* was able to resist a temperature of 73° C. and, on one occasion, even 85° C. Exps. 1, 2, 3, 5, each show that *A* had a greater heat resisting capacity than *B*.

2. In Exps. 1, 2, 3, 5, cells of *A* which were heated in milk resisted a higher temperature than those which were heated in syrup. In Exp. 4, no such difference was found; in fact, cells of *B* which had been heated in milk were killed at a temperature of 55° C., whilst those heated in syrup were still capable of growth. In this case, however, the culture used was known to be very feeble.

3. On the whole, the results indicate that *B* is less resistant to heat than *A*, and that its thermal death point is but slightly influenced by varying external conditions. On the other hand, there are indications that the thermal death point of *A* is influenced by the nature of the heating medium.

Table II.

A. (Round cells)

Exp.	Culture	Emulsion	Number of heated cells introduced into each tube	Temperatures (° C.) to which emulsions were heated					
				Unheated	55	65	73	85	100
1	Culture on B.W.A. 2 dys. at 22° C.	Milk Syrup	600,000 „	+ +	+ +	... ...	+ –	– –	– –
2	Culture on B.W.A. 9 wks. at 22° C.	Milk Syrup	800,000 „	+ +	+ +	... ...	+ –	+ –	– –
3	Culture on B.W.A. 2 dys. at 22° C.	Milk Syrup	5,400,000 „	+ +	+ +	... ...	+ –	– –	– –
4	Culture on B.W.A. 2 dys. at 22° C. 19 wks. at R.T.	Milk Syrup	12,500,000 „	+ +	+ +	... ...	– –	– –	– –
5	Culture on B.W.A. 4 dys. at 22° C. 7 dys. at 37° C.	Milk Syrup	? 2,970,000	+ +	+ +	+ –	+ –	– –	– –
6	Culture on N.A. + 60 % 4 dys. at 22° C. 7 dys. at 37° C.	Syrup	2,100,000	+	+	+	–	–	–
7	Cells on sugar 3 wks. at 30° C. 7 wks. at R.T.	Syrup	520,000	+	+	–	–	–	–

B. (Oval cells)

Exp.	Culture	Emulsion	Number of heated cells introduced into each tube	Unheated	55	65	73	85	100
1	Culture on B.W.A. 2 dys. at 22° C.	Milk Syrup	1,760,000 „	+ +	+ +	... ...	– –	– –	– –
2	Culture on B.W.A. 9 wks. at 22° C.	Milk Syrup	2,200,000 „	– –	– –	... ...	– –	– –	– –
3	Culture on B.W.A. 2 dys at 22° C.	Milk Syrup	23,400,000 „	+ +	+ +	... ...	– –	– –	– –
4	Culture on B.W.A. 2 dys. at 22° C. 19 wks. at R.T.	Milk Syrup	26,600,000 „	+ +	– +	... ...	– –	– –	– –
5	Culture on B.W.A. 4 dys. at 22° C. 7 dys. at 37° C.	Milk Syrup	? 2,100,000	+ +	+ +	– –	– –	– –	– –
6	Culture on N.A. + 60 % 4 dys. at 22° C. 7 dys. at 37° C.	Syrup	1,380,000	+	+	–	–	–	–
7	Cells on sugar 3 wks. at 30° C. 7 wks. at R.T.	Syrup	2,660,000	–	–	–	–	–	–

+ = Presence of gas. – = Absence of gas. B.W.A. = Beer-wort agar.
N.A. + 60 % = Nutrient agar + 60 % cane sugar. R.T. = Room temperature.

SUMMARY.

1. Two types of yeasts, *A*, round cells, *B*, oval cells, were isolated from a "blown" tin of sweetened condensed milk.

2. Both types were able to reproduce similar "blowing" when inoculated into tins of sweetened condensed milk.

3. The oval type was considerably more rapid and vigorous in its action than the round, but was less resistant to heat and drying.

4. These yeasts are compared with those isolated by Pethybridge from Irish, by Hammer from American, and by Knudsen from Danish and Dutch, sweetened condensed milk.

REFERENCES.

(1) BARBER, M. A. (1914). *Philippine Journal of Science*, IX, 4.
(2) HAMMER, B. W. (1919). *Iowa Agricultural Experiment Sta. Res. Bull.* No. 54.
(3) HANSEN, E. CHR. (1881). *C. R. des trav. du lab. de Carlsberg.* Book 3.
(4) KNUDSEN SØNCKE (1922). *Den. Kgl. Veterinaer-og Landbohøjskole Aarsskrift.*
(5) PETHYBRIDGE, G. H. (1906). *Econ. Proc. Roy. Dublin Soc.* I, Part 7.

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A CRITICAL ENQUIRY INTO THE ALLEGED FIXATION OF NITROGEN BY GREEN ALGAE[1]

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(With Plates XXI and XXII, and 3 Text-figures.)

I. INTRODUCTION.

Since the investigations of Lawes and Gilbert (14, 15) and of Hellriegel and Wilfarth (10) on the higher plants and of Kossowitsch (12) on the algae, it has been generally believed that green plants are not able by themselves to assimilate atmospheric nitrogen. Claims have been made from time to time, however, that certain chlorophyll-bearing organisms (*e.g.* algae, mosses and even higher plants) are able to derive their nitrogen directly from the air.

As far as algae are concerned, the literature on the subject up to 1914 has been adequately summarised and discussed by Schramm (23); a detailed consideration of this earlier work is thus rendered unnecessary in the present paper, but references to the more important of these papers are included in the bibliography (3, 7, 8, 9, 13, 16, 19, 22). The position up to and including Schramm's work may be briefly summarised as follows. The greater number of the investigations had been carried out on cultures of algae which were impure. In several of these investigations nitrogen-fixation had been established, but, in considering their bearing on the question of the ability of algae themselves to fix nitrogen, these results may be dismissed, since it is impossible to say whether the fixation was effected in part by the algae or wholly by the associated bacteria. This does not preclude the possibility that the algae are indirectly concerned in the process of nitrogen-fixation by bacteria; indeed, from the work of Kossowitsch (12) and others (1, 2, 5, 20, 21), it is extremely probable that algae and nitrogen-fixing bacteria work together in a definite association, the algae stimulating the activity of the bacteria and enabling them to fix greater quantities of nitrogen than they could do alone. Heinze (9) claimed that he had obtained evidence of nitrogen fixation in a culture of *Nostoc* contaminated with a species of *Streptothrix* which alone had given no evidence of such fixation; he therefore concluded that *Nostoc* was able to fix nitrogen. From the known difficulty of eliminating contaminating organisms from a mucilaginous envelope such as that with which *Nostoc* is always invested, it seems doubtful whether *Streptothrix* was the only such organism present.

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Turning to those investigations which had been carried out on cultures of algae of the purity of which there is little or no doubt, it is found that in no case had the results indicated a fixation of nitrogen. Schramm (23) studied a greater number of species than any earlier investigator, and his biological technique was very carefully worked out. He found that in the absence of combined nitrogen no growth took place with any of the species used, except for an "incipient ephemeral growth" which could only be detected with difficulty, and which he attributed to the unavoidable introduction of traces of combined nitrogen in the inoculum. This inability of the algae to grow in the absence of combined nitrogen, seemed to him to be conclusive evidence that they were unable, under these conditions, to assimilate free nitrogen. This conclusion one would scarcely be disposed to dispute since, in the absence of combined nitrogen, growth and fixation of free nitrogen are mutually interdependent.

Since 1914, papers on the subject have been published by Moore and his co-workers (17, 18) and most recently by Wann (25). The former claimed to have shown that fresh water and marine algae could assimilate free nitrogen. Their conclusions are, however, of doubtful validity, since the evidence that their cultures of algae were free from bacteria cannot be regarded as adequate. The elimination of bacteria from an algal culture is a matter of considerable difficulty, necessitating a special technique and taking a considerable time. Moore worked with cultures which he had made no attempt to purify, and based his conclusions as to the absence of bacteria solely on the absence of turbidity of the liquid at the end of eight days. The sheaths of algae growing under natural conditions are invariably inhabited by bacteria which, by reason of their position, do not cause any turbidity in the surrounding fluid; the absence of turbidity is therefore no criterion of the absence of bacteria. Moore, indeed, admits the possibility of the presence of bacteria in his cultures, and his arguments in favour of nitrogen-fixation by the algae rather than by the associated bacteria, if present, are unconvincing in view of the work of Kossowitsch and others.

It may therefore be fairly stated that at the time of publication of

Wann's paper (25) there was no satisfactory evidence of the ability of algae to assimilate free nitrogen, and, indeed, that all the more critical investigations had given results definitely against such a possibility.

Wann's experiments were carried out on very similar lines to those of Schramm (23), but with the important difference that combined nitrogen was intentionally included in all the media, since Schramm had failed to induce growth in its absence. By analysing his cultures at the beginning and at the end of the experiment, Wann obtained results from which he concluded that all the algae tested by him possessed the power of assimilating appreciable quantities of atmospheric nitrogen in the presence of combined nitrogen in the form of nitrate, together with glucose. The cultural technique adopted by Wann did not differ fundamentally from that of Schramm, but owing to the necessity for chemical analysis as a criterion of fixation, certain modifications had to be introduced. These consisted chiefly in the use of an agar medium instead of purified sand, and in the culture of the algae in the Kjeldahl flasks in which the analyses were ultimately to be made. Wann worked with pure cultures of seven species of algae, of which two had been used by Schramm. It is unnecessary to describe his cultural technique in more detail at this point, since that used by the present authors, to be described later, is essentially the same. The chemical methods used by Wann for the determination of the total nitrogen in his cultures are discussed in detail later.

The question of nitrogen-fixation by algae is of considerable interest from the general point of view of plant physiology; it is of still greater interest with reference to soil fertility. Of all the nutrient elements on which plants depend for growth, nitrogen is of the greatest general importance in practice; in this country crop yields are limited probably more by deficiency of nitrogen than by lack of any other single element. Any agency which helps to increase the amount of combined nitrogen in the soil may therefore be an important factor in soil fertility. The regular occurrence of algae in cultivated soils makes it very desirable that Wann's conclusions should be verified. The investigation described in the present paper was therefore undertaken.

II. EXPERIMENTAL METHOD.

A. General.

An attempt was made to reproduce as nearly as possible the conditions which obtained in Wann's experiment, as described in his paper. It was decided, however, to limit the investigation to four species of algae, growing them on six solid media selected from among those used by Wann and giving, according to him, the most striking results. The cultures were grown in Kjeldahl flasks of 500 c.c. capacity in which they could be finally subjected to chemical analysis: these were numbered consecutively from 1 to 96.

(1) *Cleaning of apparatus.* After careful washing in the ordinary way the Kjeldahl flasks were specially cleaned by projecting into each of them a jet of live steam for at least an hour, after which they were carefully rinsed with freshly distilled nitrogen-free water and dried; in this way the possibility of error by the solution of nitrogenous substances from the surface of the glass was precluded.

Erlenmeyer flasks and boiling tubes were cleaned with a strong solution of potassium dichromate in concentrated sulphuric acid, rinsed with distilled water and inverted in a steamer for three hours, finally being rinsed with nitrogen-free water and dried. Gas washing bottles were treated in the same way except that the steaming was omitted. Calcium chloride tubes were boiled for half an hour in Hudson's soap solution before being steamed. Glass tubing was cleaned successively by means of small wads of cotton-wool saturated with water and methylated spirit. Rubber tubing was fixed in a long piece to a tap and water allowed to run slowly through it for about fifteen hours. It was then cut into lengths of 1½–2 inches suitable for making the connections, and rinsed with distilled nitrogen-free water. Rubber stoppers were scrubbed and then soaked in running water for some hours: they were finally rinsed with distilled nitrogen-free water.

By these means it could be safely concluded that all nitrogenous and toxic substances had been removed from the apparatus before use.

(2) *Assembly of the apparatus.* The apparatus was set up in the laboratory in a well-lighted corner close to south and east windows, on a table covered with white American cloth to provide maximum illumination, and was supported in a specially constructed wooden stand painted white. It consisted of six parallel series of flasks, fitted together by means of bent glass tubing and rubber connections and attached at their extremities to the six side branches of two wide bored tubes, referred to subsequently as the inlet and outlet tubes. (See Plates XXI A and XXII.)

In order to ensure optimum growth of the cultures air was drawn daily through the apparatus by means of a filter pump attached to a central side branch of the outlet tube. It was drawn from outside the building through a long piece of "Compo" tubing attached to a central branch of the inlet tube and was filtered and washed in a special apparatus at the beginning of each series of flasks, as shown in Text-fig. 1A.

After passing through the calcium chloride tube *a*, filled with unsterilised cotton-wool to remove suspended particles, the air was bubbled slowly first through the wash bottle *c* containing 150 c.c. of a 25 per cent. solution of sulphuric acid, to remove traces of ammonia, and then through

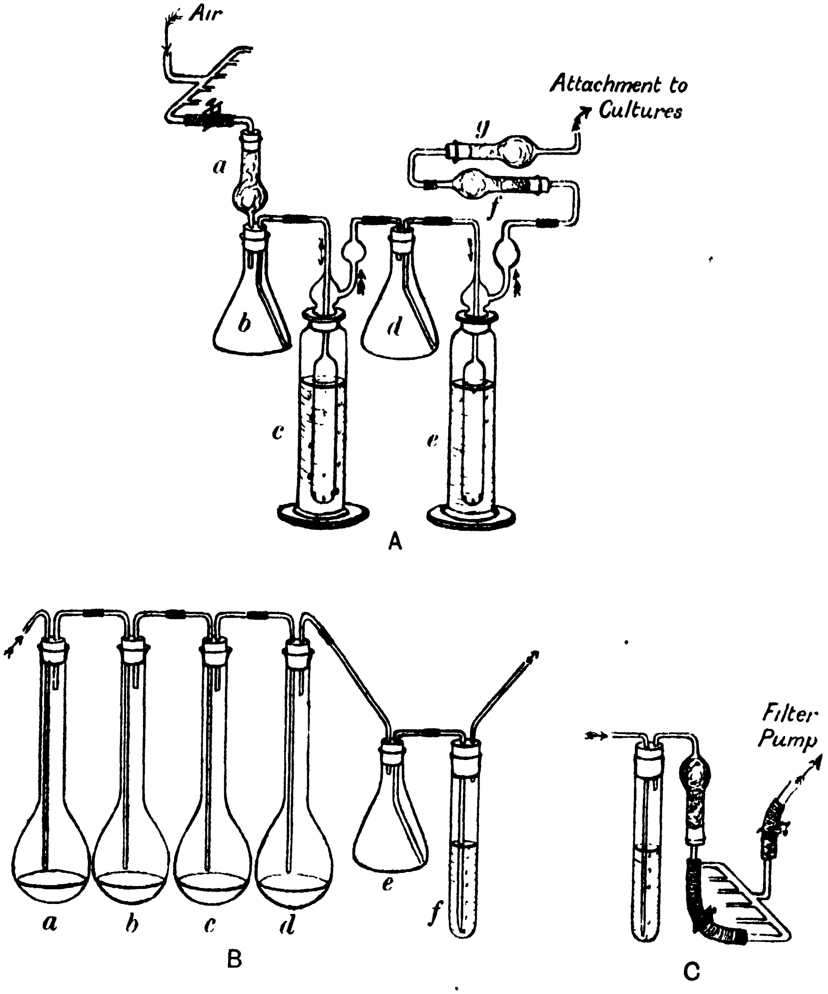

Fig. 1. Diagram showing details of apparatus. A. Apparatus for washing and filtering air, attached to 7-branched inlet tube; *a*, calcium chloride tube packed with cotton-wool; *b* and *d*, safety flasks; *c*, wash bottle containing 25 per cent. sulphuric acid solution; *e*, ditto, containing 1 per cent. sodium bicarbonate solution; *f* and *g*, as *a*, but sterile. B. A single group of sterile flasks (four in each series); *a*, uninoculated control; *b–d*, culture flasks; *e*, safety flask; *f*, boiling tube containing *N*/10 sulphuric acid. C. End of series of culture flasks attached to 7-branched outlet tube.

the wash bottle *e* containing 150 c.c. of a 1 per cent. solution of sodium bicarbonate, to remove traces of nitrous gases while still allowing the free passage of atmospheric carbon dioxide into the apparatus. It was finally passed through the two sterile calcium chloride tubes *f* and *g* filled with cotton-wool, and thus entered the series of culture flasks free from traces of nitrogenous compounds and from bacterial and fungal contaminations.

Each of the six series of flasks consisted of sixteen Kjeldahl flasks separated into four groups of four. At the end of each group was placed a boiling tube containing 50 c.c. *N*/10 sulphuric acid free from nitrogen in order to absorb any traces of ammonia that might be liberated from the media during the experiment, together with a safety flask to prevent any sucking back of the acid into the culture flasks. Each flask was provided with a two-holed rubber stopper through which passed two bent glass tubes for the passage of air through the apparatus. In the culture flasks the end of the inlet tube reached nearly to the surface of the medium to ensure that the atmosphere in the flask was changed at each aeration (see Text-fig. 1 B). Each series was terminated by a sterile calcium chloride tube filled with cotton-wool, through which the air passed before leaving the apparatus. This was included to prevent possible contamination of the cultures by the sucking back of air when the filter pump was disconnected. By means of screw clips on the pressure tubing which joined the six series of flasks to the branches of the main inlet and outlet tubes, the rate of passage of air during aeration could be regulated separately for each series, and while aeration was not proceeding each series could be completely closed off from the rest of the apparatus.

The apparatus was first completely assembled empty to ensure that all the connections were as accurately in register as possible. The stoppers fitted with their tubes were then marked, each with the number of the flask to which it belonged. The apparatus was then dismantled, the rubber connections being left attached to the inlet tubes of the flasks. Each stopper with its tubes, the upper ends of which had first been provided with paper caps, was separately wrapped up in glazed paper and the packet numbered. The *N*/10 acid was placed in the acid tubes; these and the safety flasks were then plugged with cotton-wool.

The numbered Kjeldahl flasks were provided with loops of copper wire by which they were suspended on a 2-pan balance and weighed empty. One hundred and fifty cubic centimetres of the appropriate medium were then introduced hot into each, and a cotton-wool plug inserted in the neck. As soon as the medium was cold the plug was removed for a few moments while the flask and contents were weighed. In this way the exact weight of medium in each flask was determined. Four spare Kjeldahl flasks of each medium were poured for use in the determinations of the initial nitrogen content of the medium and to provide a reserve in case of accidents; they were scattered at random through the series so as to obtain samples of medium as representative as possible of the whole. The small remaining quantities of medium were placed in Erlenmeyer flasks of 70 c.c. capacity for sub-cultures of the organisms to be used in the experiment.

B. CULTURAL.

(3) *The Media.* Wann concluded that the fixation of atmospheric nitrogen took place only in algal cultures grown on media containing nitrate in the presence of glucose, and his figures indicated a much greater fixation when all the nitrogen was present as nitrate than when, as in the case of ammonium nitrate, half the nitrogen was present as ammonia. There was no clear indication of fixation either on media in which nitrogen was supplied wholly as some compound other than a nitrate, or in the absence of glucose even when nitrate was present.

Consequently, for use in the present experiment, three of Wann's media were selected having as a common basis a non-nitrogenous mineral salts solution devised by Schramm, but differing from one another in the salt added as source of nitrogen. Each of these media was set up in two series, one without added sugar and the other containing 1 per cent. glucose. A stock solution of mineral salts was used for the whole experiment; 25 litres was made up and this sufficed for the whole of the media and for most of the preliminary chemical tests necessary to establish a suitable method of analysis of the media in the flasks.

It contained:

Magnesium sulphate ($MgSO_4$, $7H_2O$)	0·410 gm.	per litre of water distilled in a silver still.
Calcium chloride ($CaCl_2$, $6H_2O$)	0·243 gm.	
·005 per cent. solution of ferrous sulphate ($FeSO_4$, $7H_2O$)	50 c.c.	
Dipotassium hydrogen phosphate (K_2HPO_4)	0·200 gm.	

In preparing this solution it was found necessary to dissolve the potassium phosphate separately in a small quantity of water and to add it in this condition to the solution of the other salts in the rest of the water. In this way only a slight turbidity of the liquid was produced which did not settle and it was possible to obtain a uniform sample of the solution.

Of the stock solution a sufficient volume was taken, 7 litres, to make up the whole of the two media containing any one source of nitrogen;

the appropriate nitrogenous salt was dissolved in this solution which was then divided into two equal parts. To each of these was added 1·5 per cent. of washed agar and the mixture autoclaved and filtered. After filtration, 150 c.c. portions of the hot medium were measured out from one flask into twenty weighed Kjeldahl flasks which were plugged with cotton-wool and left to cool. The filtered medium in the second flask was provided with 1 per cent. glucose and thoroughly shaken before being similarly treated. The six media were thus of the following composition, with the addition of 1·5 per cent. of agar in every case:

	Flasks
I. Stock solution + ·05 % ammonium nitrate [NH_4NO_3]	1–16 + 4 extra
I A. As I + 1 % glucose	17–32 + 4 ,,
II. Stock solution + ·0942 % ammonium sulphate [$(NH_4)_2SO_4$]	33–48 + 4 ,,
II A. As II + 1 % glucose	49–64 + 4 ,,
III. Stock solution + ·1475 % calcium nitrate [$Ca(NO_3)_2 4H_2O$]	65–80 + 4 ,,
III A. As III + 1 % glucose	81–96 + 4 ,,

The amounts of the nitrogenous salts added to the media were calculated so as to give approximately equal quantities of nitrogen in all.

Owing to an unexpected delay in filling the flasks with medium III A, which necessitated its being kept hot on the water bath for some time, a further quantity of water was added to this medium. This accounts for its slightly lower nitrogen content compared with medium III.

(4) *Sterilisation.* After weighing, the flasks of medium were autoclaved at 115° for 15 minutes and placed in order in the stand: when cold, the plugs were covered with caps of tinfoil to prevent evaporation and the flasks left for two weeks before inoculation. This waiting period made it quite certain that none of the flasks contained contaminations before inoculation. The check flasks were analysed on the day on which inoculation of the culture flasks was carried out.

The rubber stoppers and glass tubes and the calcium chloride tubes filled with cotton-wool, all in their numbered paper packets, were autoclaved at 115° for 15 minutes and then dried quickly in the drying room before being stored. The acid tubes and safety flasks were similarly autoclaved and replaced in position in the stand.

(5) *Species of algae taken.* Four species of algae which had previously been obtained in pure culture[1] were selected for investigation. All four

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[1] Species A, C and D were obtained in pure culture by one of the authors working by the courtesy of Prof. R. Chodat in his laboratory at the University of Geneva, and under his direction. The grateful thanks of this author are tendered to Prof. Chodat not only for his kind hospitality but also for his invaluable help and encouragement. Species A was isolated from impure material collected at Mount St Bernard. Species C and D from a soil sample taken near Geneva.

Species B was presented in pure culture by Dr M. Denis of Clermont University, France, to whom the grateful acknowledgments of the authors are made. The material was originally obtained from a subaerial habitat.

species were unicellular green algae which for convenience may be designated by the letters A, B, C, and D. Among the lower algae the task of isolation and identification of the various closely related species is one of extreme difficulty, and it would be out of place in the present paper to go into this question in detail. It is hoped, however, shortly to publish an account of the general morphological and physiological characters of the four algae, when a more accurate designation of the species may be possible. The following particulars are sufficient for the present purpose.

Species A, *Chlorella* sp. nova affinis *Chl. coelastroides* Chod., with round or oval cells provided with a chloroplast which completely lines the cell-wall except for a single circular perforation on one side and which contains a conspicuous pyrenoid. Multiplication takes place with the formation of four to eight autospores, or more on sugary media. It differs from *Chl. coelastroides* Chod. chiefly in the form of the colonies on various media.

Species B, *Pleurococcus* sp., a very minute species with oval cells, rarely remaining attached to one another after division. This alga was described as a species of *Stichococcus* (4). It appears, however, to be more like a *Pleurococcus*, in that division has been observed in three planes and a minute pyrenoid is amost invariably present. In old cultures there is always a small proportion of larger cells in which typical protococcoid division has been observed.

Species C, *Cystococcus* sp. In young and vigorously growing cultures this species exists almost entirely in a palmelloid condition with a copious enveloping mucilage; multiplication takes place in this state by successive bipartition, but in no circumstances have zoogonidia been observed. In ageing cultures the cells become enlarged and round themselves off, assuming a typical *Cystococcus* form, in which eventually multiplication may take place by the production of autospores.

Species D, *Scenedesmus* sp. in the sense of Chodat: this designation is given tentatively pending a further investigation of the characters of the alga. It is an ill-defined species which is usually chlorelloid in form, but which in the presence of calcium nitrate and glucose exhibits a distinct tendency to form rather irregular coenobia with the cells slightly pointed and thickened at the two poles. It periodically enters into a resting state with the formation of enlarged thick-walled resting spores containing an orange pigment.

(6) *Inoculation.* Previous observations on these species grown in pure culture had demonstrated that when inoculated for the first time on to a given medium they tend to grow much more slowly and less luxuriantly

than after one or more subsequent reinoculations on to the same medium. Hence preliminary stab cultures of each of the four species were made on each of the media to be used in the experiment. For Species A, B and C sub-cultures of the second generation on each medium were obtained, but for Species D only the first sub-cultures were available. A liquid suspension of each of the cultures was made by transferring the alga by means of a sterile glass spoon from the surface of the agar to a sterile solution of nitrogen-free mineral salts, and well shaking the mixture. Hence the amount of combined nitrogen present in the small quantity of inoculum added to each flask was negligible.

In setting up the experiment, each group of Kjeldahl flasks with its accompanying acid tube and safety flask was removed separately to the inoculation chamber, which had previously been washed down with lysol. Here three of the culture flasks were inoculated each with eight to ten drops of the appropriate algal suspension while the fourth was left uninoculated as a control. Sterile capillary Pasteur pipettes, freshly drawn out, were used for making the inoculations, one for each flask. After inoculation each flask was quickly fitted with its own stopper, freshly removed from its paper wrapping, and the whole group connected together in its final form, aseptic conditions being aimed at throughout. This completed unit was then transferred to its proper position in the laboratory, the paper caps on the open ends of the tubes being left on until the units were connected together in their proper sequence. All the stoppers and rubber connections were immediately coated with melted paraffin wax in order to render all joints completely airtight.

A plan of the experiment is given in Text-fig. 2, which shows the relative positions of the control and culture flasks and of the species and media in respect to one another.

The inoculation of the cultures was carried out on three successive days, Series I and I A on May 3rd, II and II A on May 4th, III and III A on May 5th: air was drawn through the completed apparatus for the first time on the morning of May 6th, 1922.

C. CHEMICAL.

(7) *Methods of Analysis.* Although, from the biological side, Wann's work appears to be adequate, the same cannot be said of the chemical side. It is now necessary to consider this point in detail in order to show in what way the errors in Wann's analytical methods arose, how his conclusions are invalidated by these errors, and how such errors were avoided in the present investigation.

It is well known that the Kjeldahl method is not directly applicable to materials containing more than a very small proportion of their nitrogen in the form of nitrate, owing to the loss of nitric acid which occurs during the oxidation with strong sulphuric acid. Several means have been devised for overcoming this difficulty; the best known consists in a treatment of the substance to be analysed with salicylic acid or phenol dissolved in strong sulphuric acid (6, 11). By these means, working under suitable conditions, it is possible to fix the whole of the nitric acid in the form of a nitro compound, which is then reduced to an amino compound by the addition of sodium thiosulphate or zinc dust. After such treatment the digestion with strong sulphuric acid may be carried out in the usual manner until all the organic matter has been oxidised and the nitrogen is all converted to sulphate of ammonia. This method of preventing loss of nitric acid cannot, however, be used in the presence of more than traces of water, for the quantitative conversion of nitric acid to nitro compound can only be effected in concentrated sulphuric acid. The method can therefore be applied to agar media containing nitrate and a large amount of water, such as those in question, only after the removal of the bulk of that water without loss of nitrogen. The method used by Wann for the removal of water may be quoted from his paper: "A considerable part of the water of the medium was boiled off in the presence of 10 c.c. of concentrated sulphuric acid, the process being continued until active foaming began." The boiling down of the medium in the presence of 10 c.c. of strong sulphuric acid until active foaming occurred, that is, until the concentration of the sulphuric acid is high, will necessarily result in the boiling off and loss of a considerable part of the nitric acid set free by the sulphuric acid. The subsequent addition of phenol-sulphuric acid to the concentrated liquid for the purpose of fixing the nitric acid is rather in the nature of "shutting the stable door after the horse has gone."

In the absence of precise information regarding the exact method used for boiling down it is impossible to say how much nitric acid was lost in Wann's analyses. Table I shows the extent of such loss in an experiment carried out by the authors, in which two 150 gm. portions of medium containing nitrate were boiled in a Kjeldahl flask connected to a condenser, after the addition of sulphuric acid. Boiling was continued until active foaming, accompanied by charring, began. The amount of nitrate in the distillate was then determined by reduction to ammonia with Devarda's alloy.

Table I. *Loss of nitric acid caused by boiling down a nitrate medium with the addition of sulphuric acid.*

150 gm. of medium containing mineral salts, agar and 0·2000 gm. pure potassium nitrate.

Nitrate present in medium (0·2000 gm. KNO_3) mgm.	Volume of concentrated H_2SO_4 added c.c.	Nitrogen found in distillate mgm.	Nitrogen boiled off %
27·7	10	3·9	14·1
27·7	1	1·5	5·4

It is seen that in using 10 c.c. of sulphuric acid (Wann's conditions) no less than 14·1 per cent. of the nitric nitrogen originally present has been boiled off and collected in the distillate. Wann no doubt added sulphuric acid in order to prevent the loss of any ammonia from ammonium salts or organic nitrogen compounds, and it was thought possible, though unlikely, that the addition of a much smaller amount of sulphuric acid would eliminate the loss of nitric acid on boiling down. That the addition of only 1 c.c. of sulphuric acid was still fraught with a loss of 5·4 per cent. is seen from the table. The loss observed in the presence of 10 c.c. of sulphuric acid might well be much higher if boiling were continued a little longer, especially if an open flask were used.

The bearing of these results on Wann's conclusions is obvious. Not only do they render untrustworthy his analyses of media containing nitrate, but they also make it likely that the whole of his apparent fixations are illusory.

It must be borne in mind that at the end of the experiment a portion of the nitrogen originally present as nitrate would have been converted by the organisms into organic substances, in which form it would not be driven off on boiling down with sulphuric acid. In the cultures, therefore, there would be a greater amount of nitrogen immune from this loss than in the controls, so that the proportion of the nitrogen originally present which would be recovered would be greater after the growth of algae. The differences obtained when the nitrogen recovered from Wann's controls is subtracted from that recovered from his cultures, instead of being an indication of fixation, as concluded by Wann, may merely indicate that in the analyses more nitrogen had been lost from the controls than from the cultures.

The illusion of fixation is increased by the fact that this difference in recovery of nitrogen from controls and from cultures would be greater the bigger the growth of the algae in the culture, *i.e.* the greater the amount of the original nitrate converted to organic material. This interpretation of Wann's results is strengthened by the fact that he obtained apparent fixation only on those media containing nitrate, that is, only under circumstances when this analytical loss was possible, and that the apparent fixation was greater when all the nitrogen was originally present as nitrate ($Ca(NO_3)_2$) and only roughly half as much when only half the nitrogen was present as nitrate (NH_4NO_3). For all other sources of combined nitrogen there was no evidence of fixation in his results.

It should also be pointed out that whereas the agreement between the results of Wann's parallel check analyses, as reported in his paper, is usually fairly good for those media not containing nitrate, the same cannot be said in the case of media containing nitrate. In the analyses of the latter, differences up to 1·7 mg. of nitrogen per 100 gm. of medium are recorded, and it is reasonable to suppose that the differences were saved from being still greater only by the maintenance of fairly standard conditions during the boiling down of the medium.

The method used by Wann to test the trustworthiness of his method of analysis consisted in the addition of 2 gm. of agar, presumably in the solid condition, to 10 c.c. of ammonium nitrate solution, and the addition thereto of phenol sulphuric acid followed by the usual treatment. This was clearly no check on the method actually used for the media, in that the preliminary boiling down with the addition of sulphuric acid was omitted. Had a true check analysis been carried out, including all the steps used in the actual determinations, the source of error here demonstrated could hardly fail to have been detected.

It is thus possible to explain the whole of the apparent fixation observed by Wann as due to faulty analytical methods; and although it cannot be stated with certainty that there was no fixation in his experiments, it must be pointed out that his methods were not capable of demonstrating it even if it occurred, in the case of media containing nitrate. Moreover, since in the present authors' experiments no evidence of fixation could be found, using methods of analysis which were not subject to the errors inherent in those used by Wann, it is rendered unlikely that any fixation occurred in Wann's experiments.

The method of analysis used by the authors for the determination of total nitrogen in the media containing nitrate was a combination of the Ulsch method (24) with the Kjeldahl method. The nitrate in the media was converted into ammonium sulphate in acid solution, without previous concentration, by treatment with finely divided iron ("reduced iron"). This reaction takes place quantitatively with great readiness. The liquid after such treatment contains the whole of its nitrogen in the form of ammonium sulphate or organic compounds, and may then be concentrated safely and the organic matter removed by digestion with concentrated sulphuric acid in the usual way. The presence of an appreciable quantity of sulphate of iron in the reduced solution gives rise to certain difficulties. In the digestion, these are due to the separation of crystalline ferric sulphate in the strong sulphuric acid, and in the distillation, to the production of a bulky precipitate of ferric hydroxide on the addition of caustic soda. These difficulties can, however, be readily overcome by careful working. Preliminary experiments were carried out to ascertain the smallest amount of iron needed to effect quantitative reduction of the nitrate in the medium, so as to minimise the inconvenience arising from the above causes. The results of these experiments are given in Table II.

Table II. *Recovery of added nitrate in agar media.*

The medium contained mineral salts, agar, and glucose in the same amounts as the media described on p. 384. To separate 150 c.c. portions of this medium were added known amounts of pure potassium nitrate.

Amount of reduced iron used gm.	Nitrogen added as KNO_3 mgm.	Nitrogen found in 150 gm. of medium alone mgm.	Nitrogen found in 150 gm. of medium $+KNO_3$ mgm.	Nitrate nitrogen recovered mgm.	%	
2	27·3	4·8	30·5	25·7	94·2	
3	27·3	4·8	31·6	26·8	98·2	
5	27·3	4·8	31·8	27·0	99·0	Mean 100·0
5	27·3	4·8	32·1	27·3	100·0	
10	27·3	4·8	32·4	27·6	101·0	

It is seen that, working with a medium corresponding in composition to that used for the cultures and under conditions otherwise identical with those adopted in the final method (as described below), the recovery of the nitrogen of the added nitrate was incomplete with 2 gm. of reduced iron, almost complete with 3 gm. and complete with 5 gm. or more.

The method finally adopted for all media containing nitrates (*i.e.* media I, I A, III and III A) was therefore as follows: Ten cubic centimetres of 50 per cent. (by vol.) sulphuric acid (nitrogen-free[1]) were added to the medium in the Kjeldahl flask and the whole melted on the water-bath and cooled. Ten cubic centimetres of concentrated sulphuric acid were then added, followed by 5 gm. of reduced iron. The flask was fitted with a rubber stopper through which passed a calcium chloride tube filled with glass beads and coarse quartz fragments, and gently warmed. Heating was so regulated that the liquid came to the boil when the greater part of the iron had dissolved. After boiling for a few minutes the contents of the calcium chloride tube were washed into the flask, and boiling continued for a few minutes, and the tube again washed into the flask, and removed. The liquid was then boiled down until charring began. Bumping was liable to occur, and the contents of the flask often had to be kept continually shaken to facilitate regular boiling. Twenty-five cubic centimetres of strong sulphuric acid were then added and the mixture digested till frothing ceased; then a crystal of copper sulphate was added and the digestion continued till all organic matter was oxidised and the contents of the flask were light brown in colour. Any black specks in the neck were then washed down with 5 c.c. of strong sulphuric acid and the digestion continued for one hour.

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[1] The reagents used were nitrogen-free throughout.

The cooled residue was diluted with water, 110 c.c. of 50 per cent. caustic soda and a few pieces of pure granulated zinc were added, and the liquid was distilled from the Kjeldahl flask with the usual trap, through a block tin condenser, with combustion glass delivery tube. The receiver contained 25 c.c. $N/10$ sulphuric acid, and distillation was continued until at least 125 c.c. of liquid had passed over. The excess of acid was titrated with $N/10$ sodium hydroxide using methyl red as indicator, carbon dioxide being boiled off before the end point was finally determined.

III. EXPERIMENTAL RESULTS.

A. Growth Observations.

(1) *Contaminations.* As was inevitable in an experiment of this extent and complexity, a certain number of contaminations appeared in the culture flasks within a short time of the setting up of the experiment. These were dealt with in one of three ways.

(*a*) The colony of the contaminating organism was cut out with a sterile thin glass spoon, and the agar removed and weighed. This method was found to be unsatisfactory on account of the difficulty of determining the exact limits of fungal colonies, and the following was therefore adopted. (*b*) In all cases where possible, contaminated flasks were removed from the series before the infection could spread to the neighbouring flasks, and their places filled with freshly inoculated spare flasks. This method was adopted for flasks 68–72, 73 and 88. In some flasks, however, a fungal colony grew, the mycelium of which was so diffuse as completely to escape notice until considerable damage had been done, when resort had to be made to a third device. (*c*) Flask 10 was a case of this kind; the only evidence of contamination was a number of minute black dots appearing on the surface of the agar a month after inoculation. While it was still debatable whether these were actually contaminations, similar structures appeared in flasks 10 and 11, and the whole series was, as a precaution, isolated from the rest of the apparatus by means of plugs of sterile cotton-wool inserted in the glass tubes connecting flasks 9 and 10 and flask 13 and the adjacent acid tube. Contaminations appearing more than seventeen or eighteen days after the setting up of the apparatus were all treated by this method of introducing cotton-wool plugs, since it was considered inadvisable to insert fresh cultures so long after the beginning of the experiment. It proved effective in every case in preventing the spread of the infection.

In one flask, No. 27, the end of the inlet tube almost touched the surface of the agar, and algae grew in the moisture which accumulated on the end of the tube. Owing to a sucking back of the air in the series of flasks this moisture was carried over into the control flask, No. 26, which became secondarily inoculated with Species C.

In consequence of the extended duration of the experiment a few of the rubber connections became somewhat impoverished towards the end of the time, in spite of frequent applications of fresh wax. As a result of the sudden perforation of the connection preceding flask 85 during the process of aeration, air entered the apparatus directly from the laboratory without filtration and caused the contamination of flasks 85–87. At this period the experiment had already been in progress for more than five months, and on the appearance of the contaminating colonies an attempt was made, by immediate analysis of all the flasks of the group, to determine the nitrogen-content of the medium before the growth of the contaminating fungi could essentially affect its composition. The close agreement between the values obtained for all the flasks of the group indicates that this was successful. In flasks 85 and 86 the contaminations grew very rapidly, but in flask 87 there was only a single minute fleck of mycelium whose effect must have been negligible. Similar minute contaminations appeared within four days of the end of the experiment in several other flasks marked in Text-fig. 2 with an asterisk. Except in those cases indicated in the diagram by a large cross, the extent and duration of growth of the contaminating organisms were so small, however, that it is very improbable that they could have affected the main issue of the experiment, and such flasks are therefore included among those giving trustworthy results.

(2) *Growth of the Cultures.* Visible signs of growth appeared in the cultures within four to five days of inoculation on the media containing

glucose and in nine or ten days on the media without glucose. Periodic records were made of the development of the four organisms on the different media and it was observed that the triplicate cultures developed simultaneously and at the same rate. In Text-fig. 2 is indicated diagrammatically the amount of growth at the end of the experiment together with the proportion of the surface of the agar covered with the algae.

For convenience of comparison the cultures have been grouped according to their development into ten classes, as shown in the following table:

Table III.

	Degree of growth	Description of growth		Percentage recovery of nitrogen (average). See pp. 405–6
		On surface of agar	Between agar and glass	
1	Extremely luxuriant	Continuous stratum several mm. thick over whole of surface	As on surface, but rather less thick	96·72
2	Very luxuriant	Not quite continuous stratum, several mm. thick	Thinner than in 1	97·24
3	Luxuriant	Continuous stratum not more than 1 mm. thick	As in 2	96·77
4	Heavy	Dense skin-like stratum over whole surface, ¼–½ mm. thick	Slight growth	98·86
5	Very good	Similar to 4, but thinner; or rapid initial growth followed by staling of cultures after about six weeks	Very little growth	99·95
6	Good	Fairly rapid initial growth followed by early staling of cultures	No growth	99·55
7	Fairly good	Rather diffuse, thin, continuous stratum	Slight rim of growth	99·11
8	Very fair	Diffuse stratum, not continuous	Very slight growth	99·97
9	Fair	Very thin diffuse stratum, dotted with denser pin-head colonies	No growth	100·20
10	Slight	No definite stratum, but a large number of scattered pin-head colonies	No growth	99·28

In all of these classes except 9 and 10 (Species A on medium II A, Species B and C on medium III and Species D on medium I A) there was a very considerable amount of growth, enough in any case to affect profoundly the medium on which the organism was growing.

The species all attained their maximum development on media containing glucose, Species A and B on that with ammonium nitrate, and

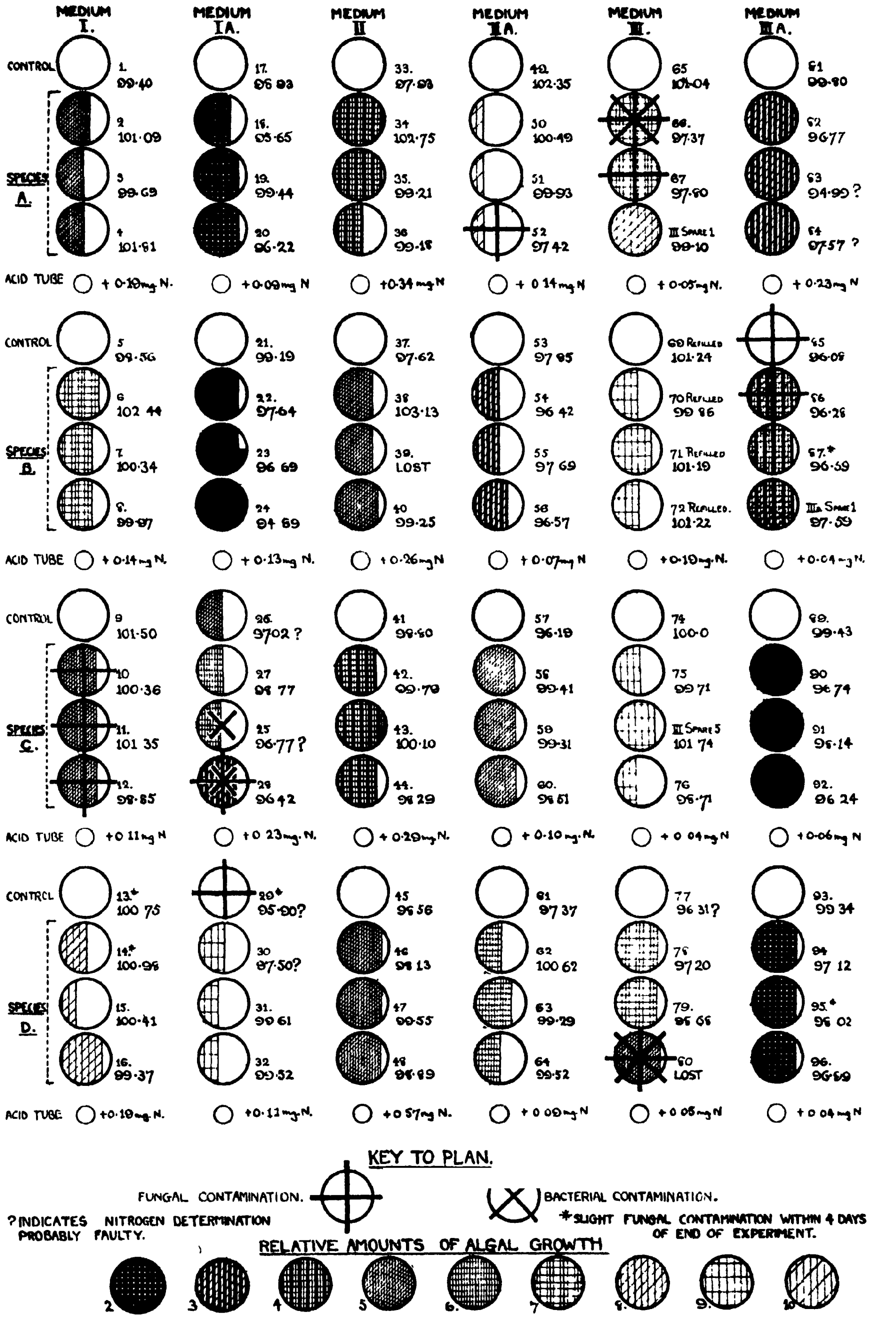

Fig. 2. Diagrammatic representation of the experiment, showing the arrangement of the media and the species and the relative amounts of growth (for explanation of latter see Table III). Of the two numbers against each flask, the upper is the serial number of the flask and the lower indicates the percentage recovery of the nitrogen originally present (for details see pp. 396–9). Similarly the amount of nitrogen found in each acid tube at the end of the experiment is shown.

Species C and D on that with calcium nitrate; in all of these cultures growth continued vigorously to the end of the experiment. Species C gave further conspicuous evidence of its physiological activity in the accumulation of large bubbles of gas, presumably oxygen given out during photosynthesis, between the agar and the glass and within the gelatinous stratum on the surface of the agar.

Species C is further noteworthy in that on media I A and II A, after a rapid initial growth, the stratum gradually lost colour until at the end of three months it had become completely colourless. No further growth took place. This phenomenon has been observed in all cultures of this organism on these media and is probably due to the accumulation of some toxic product.

Species D was conspicuous on medium III A for periodic changes in its colour. Observations on parallel cultures not included in the experiment showed these changes to be due to the periodic formation of thick-walled resting-cells containing an orange pigment. On germination these resting-cells gave rise to green vegetative cells and so caused a regeneration of the green colour of the stratum. There was no bacterial contamination in these cultures as was demonstrated by microscopic examination and by transference to a medium containing peptone and glucose.

Text-fig. 2 brings out very clearly the varying reactions of the four species to the three nitrogenous salts used, both in the presence and in the absence of glucose. Without sugar, the medium containing ammonium sulphate proved the most satisfactory for all four species, while that containing calcium nitrate was least good for three of them, A, B and C. In the presence of sugar, however, the calcium nitrate medium yielded a more luxuriant growth for the whole series than either of the other media; that containing ammonium nitrate was very good for Species A and B but failed to provide the conditions required by Species C and D.

Whether these differences in development are specific reactions of the four algae to the particular compounds supplied as source of nitrogen, or whether they are due to slight differences in the H-ion concentration of the media has not yet been determined.

At the end of the experiment each culture was examined microscopically for contamination before being analysed, and the results of these examinations are recorded in Text-fig. 2.

In almost every case the amount of algal growth in a contaminated flask was greater than that in the uncontaminated flasks of the same group, possibly on account of slightly improved conditions produced in the medium by the action of the contaminating organism.

B. ANALYTICAL RESULTS.

At the end of the experiment one complete group of flasks was removed daily, beginning on October 30th, 1922, the remaining flasks of the series being closed off by a screw clip on the rubber connection next to the group removed.

The determination of total nitrogen in each culture and control flask was always begun immediately the microscopic examination of the contents (see above) had been completed. For media I, I A, III and III A the special method described on pp. 391 and 392 was used. For media II and II A the ordinary Kjeldahl method was employed, the precautions during distillation, etc., being the same as those described in the special method just mentioned. Nitrogen was determined in the contents of the acid tubes by the micro-Kjeldahl method, rather than by direct distillation with soda, in case any organic nitrogen compounds should have been passed over into the acid.

The results of the determinations are given in the following six tables, one for each medium. Less trustworthy values are enclosed in square brackets. Reference should be made to Text-fig. 2 for details as to contaminated and doubtful analyses.

Table IV.

Medium I. Nitrogen originally present as ammonium nitrate. No glucose.

Flask No.	Species	Weight of medium, gms.	Initial nitrogen content, mgm.	Final nitrogen content, mgm.	Gain (+) or loss (−) of nitrogen, mgm.	Initial nitrogen per 100 gm. of medium, mgm.	Final nitrogen per 100 gm. of medium, mgm	Gain (+) or loss (−) of nitrogen per 100 gm. of medium, mgm.	Percentage recovery of nitrogen taken
Check	—	147·75	29·93	—	—	20·25[1]	—	—	—
”	—	148·04	30·11	—	—	20·34[1]	—	—	—
”	—	150·25	30·67	—	—	20·41[1]	—	—	--
1	Control	148·30	30·15	29·97	−0·18	20·33	20·21	−0·12	99·40
2	A	149·42	30·38	30·71	+0·33	20·33	20·55	+0·22	101·09
3		148·91	30·27	30·18	−0·09	20·33	20·27	−0·06	99·69
4		150·05	30·51	31·06	+0·55	20·33	20·69	+0·36	101·81
Acid tube	—	—	—	0·19	+0·19	—	—	—	—
5	Control	147·58	30·00	29·57	−0·43	20·33	20·04	−0·29	98·56
6	B	148·18	30·13	30·86	+0·73	20·33	20·82	+0·49	102·44
7		149·17	30·33	30·43	+0·10	20·33	20·40	+0·07	100·34
8		149·48	30·39	30·38	−0·01	20·33	20·33	0·00	99·97
Acid tube	—	—	—	0·14	+0·14	—	—	—	—
9	Control	149·29	30·15	30·60	+0·45	20·33	20·49	+0·06	101·50
10	C	148·85	30·26	[30·37]	+0·11	20·33	20·40	+0·07	[100·36]
11		147·49	29·99	[30·39]	+0·40	20·33	20·60	+0·27	[101·35]
12		148·89	30·27	[29·92]	−0·35	20·33	20·10	−0·23	[98·85]
Acid tube	—	—	—	0·11	+0·11	—	—	—	—
13	Control	147·29	29·94	30·17	+0·23	20·33	20·48	+0·15	100·75
14	D	149·45	30·38	30·68	+0·30	20·33	20·53	+0·20	100·98
15		148·67	30·23	30·35	+0·12	20·33	20·41	+0·08	100·41
16		149·00	30·29	30·10	−0·19	20·33	20·20	−0·13	99·37
Acid tube	—	—	—	0·19	+0·19	—	—	—	—
	Total	—	483·67	486·37	+2·70	—	—	—	100·6

[1] Mean = 20·33.

Table V.

Medium I A. Nitrogen originally present as ammonium nitrate, plus 1 per cent. of glucose.

Flask No.	Species	Weight of medium, gms.	Initial nitrogen content, mgm.	Final nitrogen content, mgm.	Gain (+) or loss (−) of nitrogen, mgm.	Initial nitrogen per 100 gm. of medium, mgm.	Final nitrogen per 100 gm. of medium, mgm.	Gain (+) or loss (−) of nitrogen per 100 gm. of medium, mgm.	Percentage recovery of nitrogen taken
Check	—	147·46	29·83	—	—	20·22[1]	—	—	—
”	—	149·47	30·45	—	—	20·37[1]	—	—	—
”	—	146·72	29·87	—	—	20·35[1]	—	—	—
17	Control	149·31	30·33	30·00	−0·33	20·31	20·10	−0·21	98·93
18	A	148·77	30·22	28·90	−1·32	20·31	19·43	−0·88	95·65
19		148·19	30·10	29·93	−0·17	20·31	20·20	−0·11	99·44
20		149·12	30·29	29·14	−1·15	20·31	19·55	−0·76	96·22
Acid tube	—	—	—	0·09	+0·09	—	—	—	—
21	Control	149·07	30·28	30·03	−0·25	20·31	20·14	−0·17	99·19
22	B	148·20	30·10	29·39	−0·71	20·31	19·83	−0·48	97·64
23		149·20	30·30	29·30	−1·00	20·31	19·64	−0·67	96·69
24		147·72	30·00	28·47	−1·53	20·31	19·28	−1·03	94·89
Acid tube	—	—	—	0·13	+0·13	—	—	—	—
25	C	148·11	30·08	[29·11]	−0·87	20·31	19·66	−0·65	[96·77]
26		148·13	30·09	[29·19]	−0·90	20·31	19·70	−0·61	[97·02]
27		146·99	29·85	29·48	−0·37	20·31	20·05	−0·26	98·77
28	Control	148·44	30·15	[29·07]	−1·08	20·31	19·58	−0·73	[96·42]
Acid tube	—	—	—	0·23	+0·23	—	—	—	—
29	Control	147·61	29·98	[28·75]	−1·23	20·31	19·48	−0·83	[95·90]
30	D	146·45	29·74	[29·00]	−0·74	20·31	19·80	−0·51	[97·50]
31		148·93	30·25	30·13	−0·12	20·31	20·24	−0·07	99·61
32		148·23	30·11	29·96	−0·15	20·31	20·21	−0·10	99·52
Acid tube	—	—	—	0·11	+0·11	—	—	—	—
	Total		481·87	470·41	−11·46	—	—	—	97·7

[1] Mean=20·31.

Table VI.

Medium II. Nitrogen originally present as ammonium sulphate. No glucose.

Check	—	149·12	34·39	—	—	22·99[1]	—	—	—
”	—	148·34	33·87	—	—	22·83[1]	—	—	—
”	—	148·89	34·04	—	—	22·85[1]	—	—	—
33	Control	148·47	33·99	33·28	−0·71	22·89	22·41	−0·18	97·93
34	A	148·69	34·04	34·97	+0·93	22·89	23·50	+0·61	102·75
35		148·83	33·84	33·57	−0·27	22·89	22·56	−0·33	99·21
36		146·60	33·56	33·28	−0·28	22·89	22·70	−0·19	99·18
Acid tube	—	—	—	0·34	+0·34	—	—	—	—
37	Control	148·40	33·97	33·16	−0·81	22·89	22·33	−0·56	97·62
38	B	148·43	33·98	35·04	+1·06	22·89	23·60	+0·71	103·13
39		—	—	lost	—	—	—	—	—
40		147·11	33·67	33·42	−0·25	22·89	22·72	−0·17	99·25
Acid tube	—	—	—	0·26	+0·26	—	—	—	—
41	Control	148·39	33·97	33·56	−0·41	22·89	22·61	−0·28	98·80
42	C	147·89	33·85	33·78	−0·07	22·89	22·84	−0·05	99·79
43		147·78	33·83	33·86	+0·03	22·89	22·91	+0·02	100·10
44		148·23	33·93	33·35	−0·58	22·89	22·50	−0·39	98·29
Acid tube	—	—	—	0·29	+0·29	—	—	—	—
45	Control	146·63	33·56	33·08	−0·48	22·89	22·57	−0·32	98·56
46	D	147·80	33·83	33·20	−0·63	22·89	22·47	−0·42	98·13
47		148·20	33·92	33·77	−0·15	22·89	22·79	−0·10	99·55
48		148·84	34·07	33·70	−0·37	22·89	22·65	−0·24	98·89
Acid tube	—	—	—	0·57	+0·57	—	—	—	—
	Total	—	508·01	506·48	−1·53	—	—	—	99·7

[1] Mean=22·89.

Table VII.

Medium II A. Nitrogen originally present as ammonium sulphate, plus 1 per cent. of glucose.

Flask No.	Species	Weight of medium, gms.	Initial nitrogen content, mgm.	Final nitrogen content, mgm.	Gain (+) or loss (-) of nitrogen, mgm.	Initial nitrogen per 100 gm. of medium, mgm.	Final nitrogen per 100 gm. of medium, mgm.	Gain (+) or loss (-) of nitrogen per 100 gm. of medium, mgm.	Percentage recovery of nitrogen taken
Check	—	149·59	34·05	—	—	22·76[1]	—	—	—
„	—	148·99	34·00	—	—	22·82[1]	—	—	—
„	—	149·43	34·05	—	—	22·80[1]	—	—	—
49	Control	149·19	34·00	34·80	+0·80	22·79	23·33	+0·54	102·35
50	A	149·34	34·04	34·20	+0·16	22·79	22·90	−0·11	100·49
51		149·25	34·01	33·99	−0·02	22·79	22·78	−0·01	99·93
52		147·73	33·67	[32·80]	−0·87	22·79	22·20	−0·59	[97·42]
Acid tube	—	—	—	0·14	+0·14	—	—	—	—
53	Control	149·01	33·96	33·23	−0·73	22·79	22·31	−0·48	97·85
54	B	147·77	33·68	32·47	−1·21	22·79	21·97	−0·82	96·42
55		147·51	33·62	32·84	−0·78	22·79	22·26	−0·53	97·69
56		148·61	33·88	32·72	−1·16	22·79	22·01	−0·78	96·57
Acid tube	—	—	—	0·07	+0·07	—	—	—	—
57	Control	148·72	33·89	32·60	−1·29	22·79	21·93	−0·86	96·19
58	C	148·58	33·86	33·66	−0·20	22·79	22·65	−0·14	99·41
59		149·52	34·08	33·84	−0·24	22·79	22·63	−0·16	99·31
60		149·58	34·09	33·58	−0·51	22·79	22·46	−0·33	98·51
Acid tube	—	—	—	0·10	+0·10	—	—	—	—
61	Control	149·61	34·10	33·20	−0·90	22·79	22·19	−0·60	97·37
62	D	150·02	34·19	34·40	+0·21	22·79	22·92	+0·13	100·62
63		149·16	33·99	33·75	−0·24	22·79	22·63	−0·16	99·29
64		149·34	34·01	33·87	−0·17	22·79	22·68	−0·11	99·52
Acid tube	—	—	—	0·09	+0·09	—	—	—	—
	Total	—	543·10	536·35	−6·75	—	—	—	98·8

[1] Mean=22·79.

Table VIII.

Medium III. Nitrogen originally present as calcium nitrate. No glucose.

Check	—	147·90	30·74	—	—	20·78[1]	—	—	—
„	—	148·66	30·70	—	—	20·64[1]	—	—	—
„	—	148·13	30·81	—	—	20·80[1]	—	—	—
Check (Second batch of medium)	—	149·24	31·35	—	—	21·00[2]	—	—	—
Check	—	149·46	31·40	—	—	21·10[2]	—	—	—
65	Control	149·22	30·95	31·27	+0·32	20·74	20·97	+0·23	101·04
66	A	148·31	30·76	[29·95]	−0·81	20·74	20·20	−0·51	[97·37]
67		148·64	30·83	[30·15]	−0·68	20·74	20·29	−0·45	[97·80]
Spare No. 1		148·89	30·88	30·60	−0·28	20·74	20·55	−0·19	99·10
Acid tube	—	—	—	0·05	+0·05	—	—	—	—
69 (refilled with second batch of medium)	Control	149·03	31·31	31·70	+0·39	21·01	21·27	+0·26	101·24
70	B	148·91	31·29	31·24	−0·05	21·01	20·98	−0·03	99·86
71		149·20	31·35	31·72	+0·37	21·01	21·26	+0·25	101·19
72		148·12	31·12	31·50	+0·38	21·01	21·27	+0·26	101·22
Acid tube	—	—	—	0·19	+0·19	—	—	—	—
74	Control	149·49	31·00	31·00	0·00	20·74	20·74	0·00	100·00
75	C	147·58	30·61	30·52	−0·09	20·74	20·68	−0·06	99·71
Spare No. 5 (Second batch of medium)		148·63	31·23	31·77	+0·54	21·01	21·38	+0·37	101·74
76		148·49	30·80	30·40	−0·40	20·74	20·47	−0·27	98·71
Acid tube	—	—	—	0·04	+0·04	—	—	—	—
77	Control	148·93	30·89	[29·75]	−1·14	20·74	20·00	−0·74	[96·31]
78	D	148·81	30·86	30·00	−0·86	20·74	20·16	−0·58	97·20
79		148·04	30·70	30·30	−0·40	20·74	20·46	−0·28	98·68
80		—	—	lost	—	—	—	—	—
Acid tube	—	—	—	0·05	+0·05	—	—	—	—
	Total	—	464·58	462·20	−2·38	—	—	—	99·5

[1] Mean=20·74. [2] Mean=21·01.

Table IX.

Medium III A. Nitrogen originally present as calcium nitrate; plus 1 per cent. of glucose.

Flask No.	Species	Weight of medium, gms.	Initial nitrogen content, mgm.	Final nitrogen content, mgm.	Gain (+) or loss (−) of nitrogen, mgm.	Initial nitrogen per 100 gm. of medium, mgm.	Final nitrogen per 100 gm. of medium, mgm.	Gain (+) or loss (−) of nitrogen per 100 gm. of medium, mgm.	Percentage recovery of nitrogen taken
Check	—	148·28	29·17	—	—	19·66[1]	—	—	—
„	—	148·68	29·59	—	—	19·89[1]	—	—	—
„	—	148·26	29·13	—	—	19·63[1]	—	—	—
81	Control	148·81	29·36	29·30	−0·06	19·73	19·70	−0·03	99·80
82	A	148·49	29·30	28·35	−0·95	19·73	19·20	−0·53	96·77
83		148·07	29·22	[27·75]	−1·47	19·73	18·73	−1·00	[94·99]
84		148·58	29·32	[28·60]	−0·72	19·73	19·25	−0·48	[97·57]
Acid tube	—	—	—	0·23	+0·23	—	—	—	—
85	Control	117·97	29·20	[28·05]	−1·15	19·73	18·95	−0·78	[96·08]
86	B	148·46	29·29	[28·20]	−1·09	19·73	19·00	−0·73	[96·28]
87		148·50	29·30	28·30	−1·00	19·73	19·05	−0·68	96·59
Spare No. 1		146·99	29·00	28·30	−0·70	19·73	19·25	−0·48	97·59
Acid tube	—	—	—	0·04	+0·04	—	—	—	—
89	Control	147·82	29·17	29·00	−0·17	19·73	19·63	−0·10	99·43
90	C	148·54	29·31	28·35	−0·96	19·73	19·10	−0·63	96·74
91		147·71	29·14	28·60	−0·54	19·73	19·37	−0·36	98·14
92		148·51	29·30	28·20	−1·10	19·73	18·98	−0·75	96·24
Acid tube	—	—	—	0·06	+0·06	—	—	—	—
93	Control	148·47	29·29	29·10	−0·19	19·73	19·60	−0·13	99·34
94	D	146·12	28·83	28·00	−0·83	19·73	19·15	−0·58	97·12
95		146·60	28·92	28·35	−0·57	19·73	19·35	−0·38	98·02
96		148·41	29·28	28·40	−0·88	19·73	19·15	−0·58	96·99
Acid tube	—	—	—	0·04	+0·04	—	—	—	—
	Total	—	467·23	455·22	−12·01	—	—	—	97·4

[1] Mean = 19·73.

IV. DISCUSSION OF RESULTS.

A careful examination of the above analytical results reveals the fact that in only 21 cases out of a total of 94[1] is the figure for the amount of nitrogen recovered greater than that for the nitrogen originally in the medium; moreover, this excess is in all cases less than 0·75 mg. per 100 gm. of medium, and in all cases but three less than 0·5 mg. In a protracted and complicated experiment of this kind, it is almost impossible to estimate accurately the probable experimental error[2]. From the analytical figures given on p. 391, it is seen that in three determinations on the same medium carried out almost simultaneously the extreme difference between two determinations is 0·6 mg. for 150 gm. of medium, *i.e.* 0·4 mg. per 100 gm. of medium. Similarly, the triplicate check analyses on the media actually used (see Tables IV–IX) exhibit extreme variations up to 0·26 mg. N per 100 gm. of medium. In the case of a difference between two determinations carried out at an interval of several months, during which the flasks containing the media had been subjected to the various operations in the experiment, the probable error would certainly be greater than that of three parallel determinations made together[1].

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[1] Two analyses were lost through accidents.

[2] It is, however, possible to test the agreement between the results of analyses of the contents of inoculated and control flasks with some accuracy, as shown later.

The largest gains of nitrogen found are so little greater than this probable error that the present authors regard them as not significant. In support of this it may be pointed out that:

(1) The flasks which yielded these apparent gains were scattered throughout the whole apparatus.

(2) The apparent gains were obtained for control flasks as well as for inoculated ones.

(3) Against these apparent gains must be reckoned a greater number of apparent losses.

(4) There is no correlation between the amount of growth in the flasks and the apparent gains; indeed, the most luxuriant cultures gave apparent losses in all cases (see below).

The detailed analytical results in Tables IV–IX are summarised in Table X, which gives the mean percentage recovery of the nitrogen originally taken, for each set, for each medium as a whole, for each species as a whole, and for the whole experiment, the control flasks being averaged separately from the cultures. Certain of the figures in Tables IV–X are known to be less trustworthy than the rest owing to contaminations or the probability of errors in the analyses. Such figures are enclosed in square brackets, and in Table X, where accompanied by a second figure not in square brackets, the latter figure has been obtained by the omission from the calculation of all dubious values.

Taking the experiment as a whole, and excluding doubtful cases, it will be seen that the figures for the mean percentage recovery from controls and from cultures are practically identical (99·25 and 99·28). Moreover, the mean value for each medium, and especially for each species, is only slightly divergent from these figures. The mean values for the separate groups are somewhat more variable but not conspicuously so. The same is true if all the values, including doubtful cases, are taken, the omission of the latter having little influence on the figure obtained. There is thus not the slightest evidence of fixation in these results. That the whole of the differences obtained (with the possible exception of those which, as shown below, may indicate a loss of nitrogen) are probably entirely due to experimental error is indicated by Text-fig. 3. In this figure are four distribution curves, constructed from the values for the percentage recovery of nitrogen in the separate flasks. Curves *A* and *B* have been derived from the trustworthy values for controls and for

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[1] See footnote on p. 404.

Table X. *Summary of analytical results.*

Species	Nitrogen		Medium												Total for species	
			I		I A		II		II A		III		III A			
			mgm.	Re-covery %	mgm.	Re-covery %	mgm.	Re-covery %	mgm.	Re-covery %	mgm.	Re-covery %	mgm.	Re covery %	mgm.	Re-covery %
A	In Control	Taken	30·15	99·4	30·33	98·9	33·99	97·9	34·00	102·4	30·95	101·0	29·36	99·8	188·78	99·9
		Found	29·97		30·00		33·28		34·80		31·27		29·30		188·62	
	In Cultures	Taken	91·16	101·1	90·61	97·2	101·44	100·7	[101·72] 68·05	[99·4] 100·4	[92·47] 30·88	[98·1] 99·3	[87·84] 29·30	[96·7] 96·8	[565·24] 411·44	[98·9] 99·6
		Found[1]	92·14		88·06		102·16		[101·13] 68·33		[90·75] 30·65		[84·93] 28·35		[559·17] 409·69	
B	In Control	Taken	30·00	98·6	30·28	99·2	33·97	97·6	33·96	97·9	31·31	101·2	29·20	[96·1]	[188·72] 159·52	[98·4] 98·9
		Found	29·57		30·03		33·16		33·23		31·70		[28·05]		[185·74] 157·69	
	In Cultures	Taken	90·85	101·1	90·40	96·6	67·65[2]	101·6	101·18	97·0	93·76	101·0	87·59	[96·9]	[531·43] 443·84	[98·9] 99·3
		Found[1]	91·81		87·29		68·72[2]		98·10		94·65		[84·84]		[525·45] 440·57	
C	In Control	Taken	30·15	101·5	30·15	[96·4]	33·97	98·8	33·89	96·2	31·00	100·0	29·17	99·4	[188·33] 158·18	[98·7] 99·1
		Found	30·60		[29·07]		33·56		32·60		31·00		29·00		[185·83] 156·76	
	In Cultures	Taken	90·52	[100·3]	[90·02] 29·85	[97·8] 99·5	101·61	99·7	102·03	99·2	92·64	100·1	87·75	97·1	[564·57] 413·88	[99·0] 99·1
		Found[1]	[90·79]		[88·01] 29·71		101·28		101·18		92·73		85·21		[559·10] 410·11	
D	In Control	Taken	29·94	100·8	29·98	[95·9]	33·56	98·6	34·10	97·4	30·89	[96·3]	29·29	99·3	[187·76] 126·89	[98·0] 98·9
		Found	30·17		[28·75]		33·08		33·20		[29·75]		29·10		[184·05] 125·55	
	In Cultures	Taken	90·90	100·5	[90·10] 60·36	[99·0] 99·7	101·82	99·4	102·22	99·9	61·56[2]	98·0	87·03	97·4	[533·63] 503·89	[99·1] 99·2
		Found[1]	91·32		[89·20] 60·20		101·24		102·11		60·35[2]		84·79		[529·01] 500·01	
Total for medium															GRAND TOTAL	
	In Control	Taken	120·24	100·1	[120·74] 60·61	[97·6] 99·0	135·49	98·2	135·95	98·4	[124·15] 93·26	[99·7] 100·8	[117·02] 87·82	[98·7] 99·5	[753·59] 633·37	[98·8] **99·25**
		Found	120·31		[117·85] 60·03		133·08		133·83		[123·72] 93·97		[115·45] 87·40		[744·24] 628·62	
	In Cultures	Taken	[363·43] 272·91	[100·7] 100·9	[361·13] 271·22	[97·6] 97·8	372·52	100·2	[407·15] 373·48	[98·8] 99·0	[340·43] 278·84	[99·4] 99·8	[350·21] 204·08	[97·0] 97·2	[2194·97] 1773·05	[99·0] **99·28**
		Found[1]	[366·06 275·27		[352·56] 265·26		373·40		[402·46] 369·66		[338·48] 278·38		[339·77] 198·35		[2172·73] 1760·38	

N.B. Less trustworthy values are enclosed in square brackets. See p. 401; also Text-fig. 2 for details.

[1] Including acid tubes.

[2] Two cultures only.

cultures respectively; curve *C* is a combination of curves *A* and *B*, while curve *D* contains in addition those less trustworthy values excluded from curve *C*. It will be seen that within the limits of the available data, the curves are all of the normal type, with a modal value of 98·5–99·5. In other words, the mean value for the percentage recovery of nitrogen, taking the experiment as a whole (99·25–99·28, see Table X), has a real meaning.

A statistical analysis of the data from which these curves were constructed[1] gives the following results. The 20 control values in curve *A* are scattered with a standard deviation of 1·55 per cent., that of the 59 values from inoculated flasks in curve *B* being 1·82 per cent.; the difference between these values is not significant, and together they give an average of 1·76 per cent. From this value the standard error of the difference is calculated from the formula $1{\cdot}76\sqrt{\frac{1}{20}+\frac{1}{59}} = 0{\cdot}455$. The mean of the control values is 99·30, while that of the values from inoculated flasks is 99·03, a slightly lower value; the difference, 0·27 ± 0·45, is definitely insignificant, although it is apparent that the method is sufficiently accurate to detect with certainty a difference of only 1 per cent. It should be pointed out that the above mean of the values from inoculated flasks, 99·03, is slightly lower than the percentage recovery

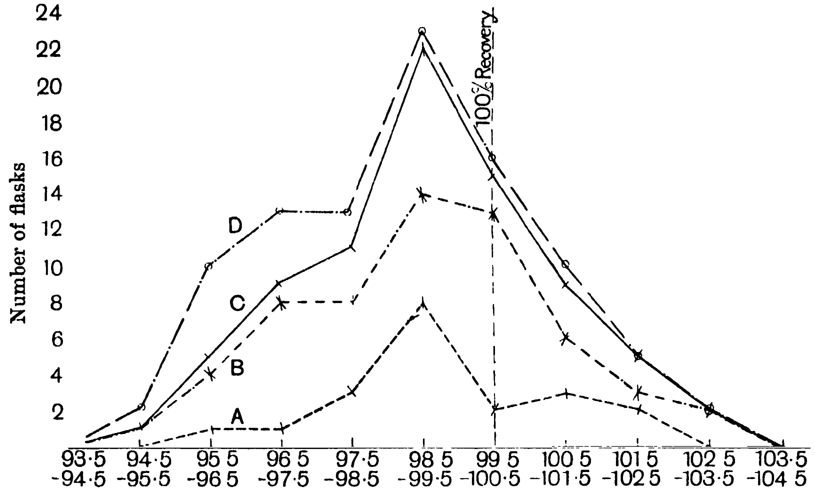

Fig. 3. Curves showing distribution of values obtained for percentage recovery of nitrogen. For description see text.

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[1] We are indebted to Mr R. A. Fisher, Chief Statistician at this Station, for this analysis.

calculated from the total of all trustworthy values, 99·28 (Table X), largely owing to the inclusion in the latter total of the nitrogen recovered from the acid tubes.

That the values for the percentage recovery of nitrogen are practically the same for controls and for cultures is considered to be the strongest evidence that no fixation has occurred. The fact that the values are slightly less than 100 can only indicate that in the analyses carried out at the end of the experiment the conditions used were not quite identical with those used for the check analyses six months or more earlier, although there was no obvious difference in any detail[1]. It is difficult to suppose that a real loss of nitrogen from the medium in the control flasks could have occurred.

The smallest percentage recoveries were obtained with Species A on medium I A and medium III A, with Species B on medium I A and medium II A and with Species C and D on medium III A. It is interesting to note (1) that these are the cultures in which growth was most luxuriant, although, had fixation occurred, they are those in which one would have expected it to be most marked; (2) that the medium in each case contained sugar; and (3) that in five of the six cases nitrogen was present in the form of nitrate. This might be taken to indicate that on media containing sugar vigorous algal growth results in a loss of gaseous nitrogen. The distribution of the values for the percentage recovery of nitrogen when the cultures are classified according to the amount of algal growth lends some support to this view. The averages of the percentage values for the cultures in each class are shown in Table III; and it is conspicuous that the averages for classes I–III (96·72, 97·24, 96·77) are considerably below the mean value for the control flasks (99·25) while the averages for classes IV–X (98·86, 99·95, 99·55, 99·11, 99·97, 100·20, 99·28) are grouped fairly regularly though slightly above this mean. When all the flasks of classes I–III are grouped separately from the rest, their percentage values are seen (Table XI, column 1) to have a fairly normal distribution about a modal value of 96·66–97·0 per cent. The flasks of classes IV–X, on the other hand, are distributed about a modal value of 99·33–99·66 (Table XI, column 2) with a range entirely comparable to that of the control flasks (column 3). The means of the values in columns 1 and 2 are 96·93 and 99·61 respectively. The difference between these, 2·65 ± 0·5, is thus significant and appears to indicate that there was a small but definite loss of nitrogen from the luxuriant cultures.

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[1] Attention has been called by "Student" (*Biometrika*, 1917, XI, 415) to the possibility of a secular error in a series of determinations not all carried out on the same date. He says: "After considerable experience I have not encountered any determination which is not influenced by the date on which it is made, from this it follows that a number of determinations of the same thing made on the same day are likely to lie more closely together than if the repetitions had been made on different days." It is quite possible that the probable error of the difference between the nitrogen-content of the flasks at the end of the experiment and that of the check flasks analysed more than six months earlier is influenced by such a secular error. Some idea of the probable magnitude of such error is given by the result of the analysis of a spare flask of medium III which had remained untouched until October, 1923, *i.e.* 17 months in all. The value for the nitrogen-content of this flask was 21·16 mgm. nitrogen per 100 gm. of medium, compared with the value of 21·01 mgm. found at the beginning of the experiment, a difference of 0·15 mgm. This would appear to indicate that the secular error due to the interval elapsing between the check analyses and the final analyses six months later is almost negligibly small.

Table XI. *Distribution of percentage values for recovery of nitrogen in relation to amount of algal growth.*

	Numbers of flasks		
Percentage values	(1) Luxuriant growth, Classes I–III. (See Table III)	(2) Heavy to slight growth, Classes IV–X. (See Table III)	(3) No growth, Controls
94·66– 95·00	1	.	.
95·00– 95·33	.	.	.
95·33– 95·66	1	.	.
95·66– 96·00	.	.	.
96·00– 96·33	11	1	1
96·33– 96·66	111	1	.
96·66– 97·00	1111	.	.
97·00– 97·33	1	1	.
97·33– 97·66	1	111	11
97·66– 98·00	1	1	11
98·00– 98·33	11	11	.
98·33– 98·66	.	1	11
98·66– 99·00	.	11111	11
99·00– 99·33	.	111111	1
99·33– 99·66	1	111111	111
99·66–100·00	.	111111	1½
100·00–100·33	.	1	½
100·33–100·66	.	11111	.
100·66–101·00	.	1	1
101·00–101·33	.	111	11
101·33–101·66	.	1	1
101·66–102·00	.	11	.
102·00–102·33	.	.	.
102·33–102·66	.	1	1
102·66–103·00	.	1	.
103·00–103·33	.	1	.

In this connection it should be noted that Wann records a similar though much greater loss with one species of *Chlorella* when nitrogen was present as ammonium nitrate or calcium nitrate. The present authors, however, are not prepared, on the basis of the comparatively limited data available to regard the existence of a denitrifying action of this type as definitely established[1].

V. SUMMARY AND CONCLUSIONS.

The work described in the present paper was undertaken in order to test the results obtained by Wann, on the strength of which this author claimed that green algae were capable, in the presence of nitrate and glucose, of assimilating free nitrogen from the atmosphere.

The present authors working with four species of green algae in pure culture, under conditions as nearly as possible identical with those used by Wann, have entirely failed to corroborate his results, although the amount of growth obtained in the cultures was in almost all cases considerable, and in some luxuriant. The average percentage recovery of nitrogen for the whole experiment was for the cultures 99·28 per cent., and for the controls 99·25 per cent.

Moreover, the method of chemical analysis used by Wann for media containing nitrates is shown to be entirely unreliable, and likely to yield figures which would give the appearance of fixation even when none had occurred. Since it was only on media containing nitrate, and thus liable to give faulty results, that Wann claimed to have obtained definite fixation of nitrogen, his results must be regarded as entirely inconclusive.

The chemical methods used by the present authors were free from the serious sources of error in those used by Wann. While it is quite conceivable that green algae might assimilate atmospheric nitrogen under certain conditions as yet unknown, there is so far no trustworthy evidence that they can do so.

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[1] A negative correlation between intensity of growth of algae and percentage recovery of nitrogen would be caused if the complex nitrogen compounds built up by the algae were not entirely broken down to sulphate of ammonia by the digestion with sulphuric acid in the Kjeldahl process. This possibility was investigated by carrying out determinations of nitrogen in dried algal material by the Kjeldahl method and by the absolute (Dumas) method. The percentages of nitrogen found were: Kjeldahl method 3·52 per cent., Dumas method 3·56 per cent. These results appear to rule out the above possibility, the difference between them being less than the experimental error of the determinations on the small amount of material available for analysis. These determinations were kindly carried out by Mr G. C. Sawyer.

SELECTED BIBLIOGRAPHY.

(1) BOUILHAC, R. (1896). Sur la fixation de l'azote atmosphérique par l'association des algues et des bactéries. *Comptes Rendus*, CXXIII, 828–30.

(2) BOUILHAC et GIUSTINIANI (1903). Sur une culture de sarrasin en présence d'un mélange d'algues et de bactéries. *Comptes Rendus*, CXXXVII, 1274–76.

(3) CHARPENTIER, P. G. (1903). Alimentation azotée d'une algue—Le *Cystococcus humicola*. *Ann. Inst. Pasteur*, 17, 321–334.

(4) DENIS, M. (1920). L'optimum lumineux pour le développement du *Stichococcus bacillaris*, Naeg. *Rev. Gén. de Bot.* XXXII, 72.

(5) FISCHER, HUGO (1904). Ueber Symbiose von Azotobacter mit Oscillarien. *Centralbl. f. Bakt.* XII, 267–8.

(6) FÖRSTER, O. (1889). Zur Bestimmung des Salpetersäurestickstoffes nach Kjeldahl'sche Methode. *Chem. Zeit.* XII, 1555.

(7) FRANK, B. (1889). Ueber den experimentallen Nachweis der Assimilation freien Stickstoffs durch Erdbewohnende Algen. *Ber. der d. bot. Ges.* Bd. VII, 34–42.

(8) GAUTIER et DROUIN (1888). Recherches sur la fixation de l'azote par le sol et les végétaux. *Comptes Rendus*, CVI, 1174–76.

(9) HEINZE, B. (1906). Ueber die Stickstoffassimilation durch niedere Organismen *Landw. Jahrb.* XXXV, 889–910.

(10) HELLRIEGEL und WILFARTH (1888). Untersuchungen über die Stickstoffnahrung der Gramineen und Leguminosen. *Zeitsch. des Vereins f. d. Rübenzucker-Industrie.*

(11) JODLBAUR, M. (1886). Die Bestimmung des Stickstoffs in Nitraten nach der Kjeldahl'schen Methode. *Chem. Zentrbl.* p. 433.

(12) KOSSOWITSCH, P. (1894). Untersuchungen über die Frage, ob die Algen freien Stickstoff fixiren. *Bot. Zeit.* V, 98–116.

(13) KRÜGER, W. und SCHNEIDEWIND (1900). Sind niedere chlorophyllgrüne Algen imstande, den freien Stickstoff der Atmosphäre zu assimilieren und den Boden an Stickstoff zu bereichern? *Landw. Jahrb.* XXIX, 771–804.

(14) LAWES, GILBERT and PUGH (1861). On the Source of the Nitrogen of Vegetation with Special Reference to the Question whether Plants Assimilate Free or Uncombined Nitrogen. *Phil. Trans.* CLI, 431–577. See also *Phil. Trans.* (1889) CLXXX, 1–107.

(15) LAWES and GILBERT. The Sources of the Nitrogen of our Leguminous Crops. *Journ. Roy. Agric. Soc.* Ser. 3, II, 657–702.

(16) MAMELI, E. e POLACCI, G. (1911). Su l' assimilazione diretta dell' azoto atmosferico libero nei vegetali. *Atti Instit. Bot. Pavia*, XV, 159–257.

(17) MOORE, BENJAMIN and WEBSTER, T. ARTHUR (1920). Studies of photosynthesis in fresh-water algae. I. The fixation of both Carbon and Nitrogen from the atmosphere to form organic tissue by the green plant cell. *Proc. Roy. Soc.* B. XCI, 201–215

(18) MOORE, WHITLEY and WEBSTER (1921). Studies of Photosynthesis in Marine Algae. I. Fixation of Carbon and Nitrogen from Inorganic Sources in Sea Water. II. Increase of Alkalinity of Sea Water as a Measure of Photosynthesis. *Proc. Roy. Soc.* B. XCII, 51–60.

(19) PRANTL (1889). Die Assimilation freien Stickstoffs und der Parasitismus von Nostoc. *Hedwigia*, XXVIII, 135–6.

(20) REINKE, J. (1903). Symbiose von Volvox und Azotobacter. *Ber. der d. bot. Ges.* Bd. XXI, 481.

(21) —— (1903). Die zur Ernährung der Meeresorganismen disponiblen Quellen an Stickstoff. *Ber. der d. bot. Ges.* Bd. XXI, 371.

(22) Schloesing fils, et Laurent, E. (1892). Recherches sur la fixation de l'azote libre par les plantes. *Ann. de l'Institut Pasteur*, vi, 65–115.

(23) Schramm, J. R. (1914). The Relation of certain Grass Green Algae to Elementary Nitrogen. *Ann. Mo. Bot. Gard.* i, 2.

(24) Ulsch, K. (1891). Die Überführung der freien Salpetersäure in Ammoniak durch Wasserstoff *in statu nascendi. Zeitsch. anal. Chem.* xxx, 175.

(25) Wann, F. B. The fixation of nitrogen by green plants. *Amer. Journ. Bot.* 1921, viii, 1–29.

EXPLANATION OF PLATES XXI AND XXII

Plate XXI a. General view of the complete apparatus, showing the air washing and filtering apparatus and the flasks of the first series.

Plate XXI b. A single unit of the experiment (Species D on Medium III a). Note the luxuriant growth in the three inoculated flasks.

Plate XXII. End view of the apparatus, showing the six series of flasks and their connections with the outlet tube.

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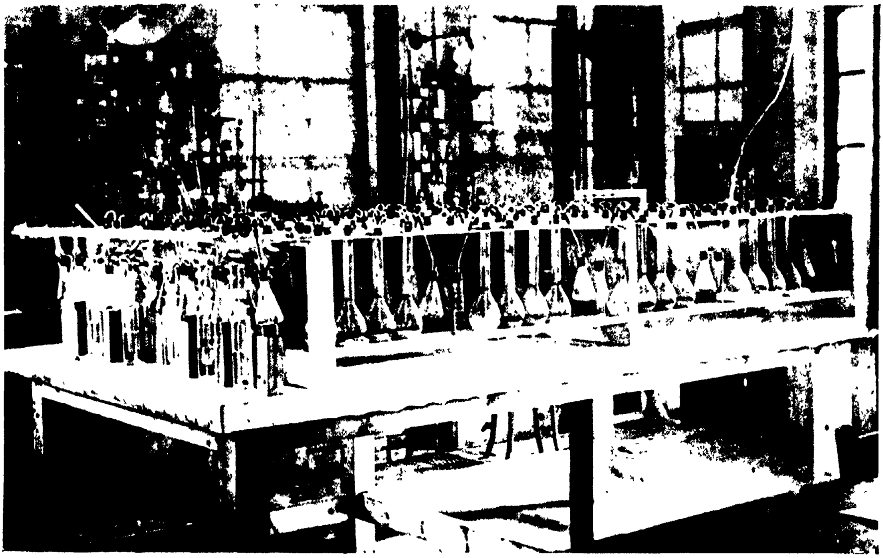

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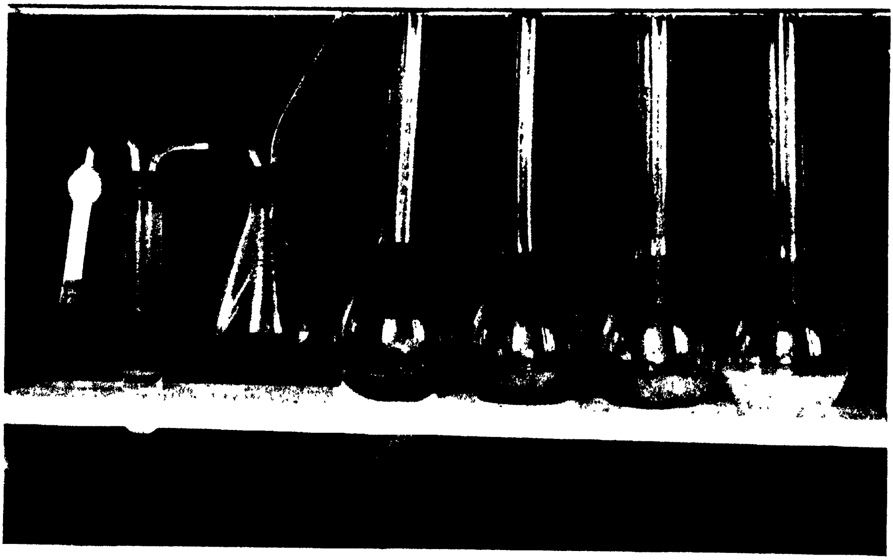
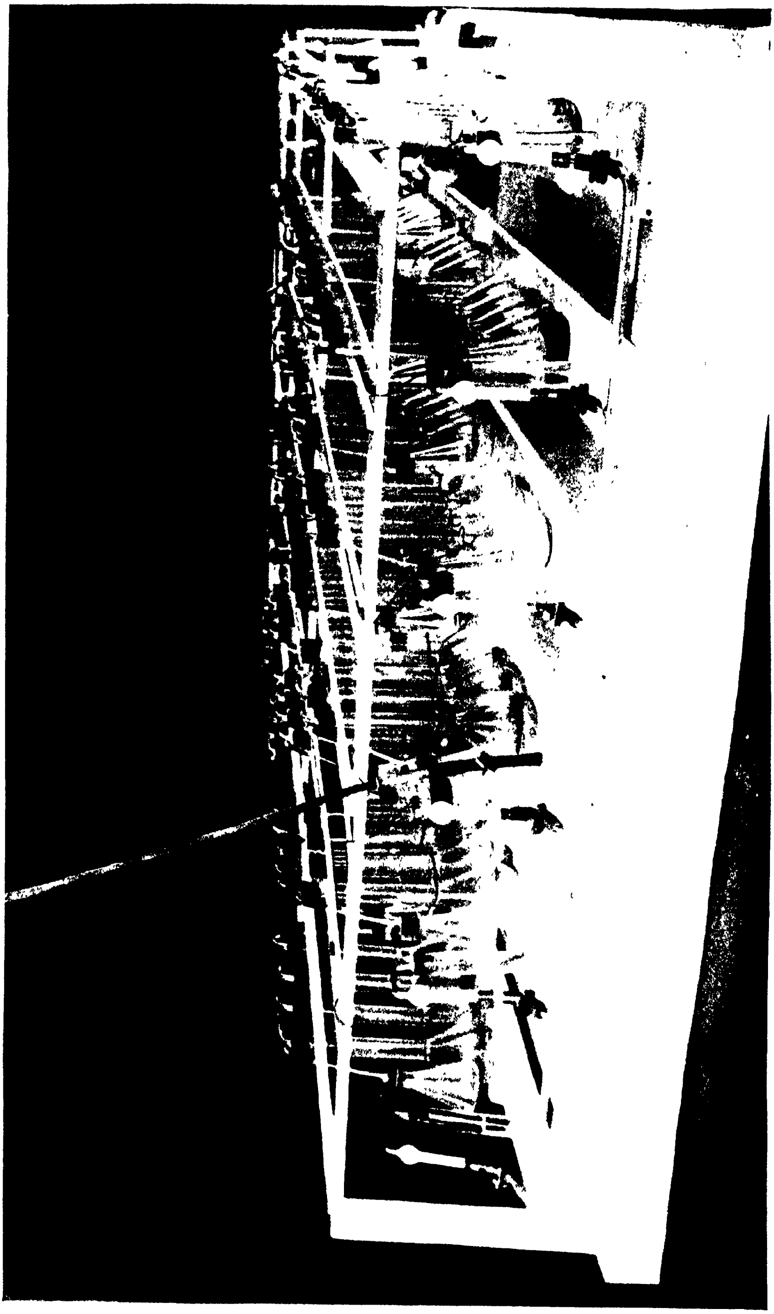


B

BRISTOL AND PAGE.—A Critical Enquiry into the Alleged Fixation of Nitrogen by Green Algae. (pp. 378—408.)



BRISTOL AND PAGE.—A CRITICAL ENQUIRY INTO THE ALLEGED FIXATION OF NITROGEN BY GREEN ALGAE. (pp. 378—408.)

ON THE LARVAL AND PUPAL STAGES OF *FORCIPOMYIA PICEUS* WINN.[1]

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(With 14 Text-figures.)

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INTRODUCTION.

THE following investigation was commenced at Birmingham University in 1921 under the supervision of Prof. F. W. Gamble, F.R.S., and was continued at Rothamsted under the direction of Dr A. D. Imms. To

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[1] A grant in aid of publication has been received for this communication.

Professor Gamble and to Dr Imms I am indebted for assistance in many ways during the course of this work. For the identification of the imagines I have to thank Mr F. W. Edwards.

The larvae were collected from beneath the bark of dead Conifers (*Larix europaea*) in Sutton Park, Birmingham, and were easily reared upon pieces of bark kept in a moist chamber. Until pupation they were reared in darkness, but were examined each day and the pupae transferred to shallow glass vessels in which they could be observed more easily. The light did not appear to affect them adversely, though under natural conditions they always pupate beneath the bark. The pupal period is from eight to ten days under laboratory conditions. In the laboratory pupation occurred towards the end of February, the imagines emerging during the early part of March. As pupae and empty pupal skins were found at approximately the same times in the open, the artificial laboratory conditions do not appear to affect the times of metamorphosis very greatly.

The larvae to be sectioned were fixed in either Gilson's or Bouin's fluid and embedded in wax with a melting point of 54° C. All sections were cut at 8μ.

EXTERNAL ANATOMY OF THE LARVA.

The larva (Text-fig. 1) consists of a well-defined head which is not capable of invagination within the prothorax, and twelve clearly marked body segments, the first and last of which bear ventrally the prothoracic and anal pseudopods respectively. The segments gradually diminish in size posteriorly.

The Head (Text-fig. 2) is a somewhat pear-shaped body bearing on its narrow ventral end the slit-like mouth. It is attached to the first body segment by a flexible lightly chitinized membrane, similar to the membrane joining adjacent segments of the body. The head capsule is formed of smooth firm chitin and bears the eyes, antennae and mouth parts and a number of prominent setae. The antennae (*a*) are situated dorso-laterally in the region where the dorsal and anterior surfaces meet. Each is two-jointed, consisting of a short and broad obtusely conical base from whose apex arises the second joint. The second joint is rod-like and tapers to a blunt apex bearing a minute stiff peg. The eyes (*E*, Text-figs. 1 and 2) are small, circular, and very convex protuberances of transparent chitin.

The vertex of the head bears near its posterior margin a pair of long curved setae projecting dorsally and anteriorly (*p.s.*). Their bases are separated by about two-thirds of the width of the head. They bend first slightly towards each other and then strongly outwards, so that their apices are separated by considerably more than the width of the head. The antennae arise anteriorly and slightly laterally to the bases of these setae. Anterior to the antennae and more medianly situated, is a second pair of dorsal setae (*a.s.*). These project forwards and then curve downwards and outwards. Midway between these and the anterior border of the mouth, the anterior surface of the head bears a pair of frontal setae which diverge from their bases and curve downwards (*f.s.*). The head bears two setae on each side, one of which is much shorter than the other. The short seta is situated postero-ventrally to the antenna, projects

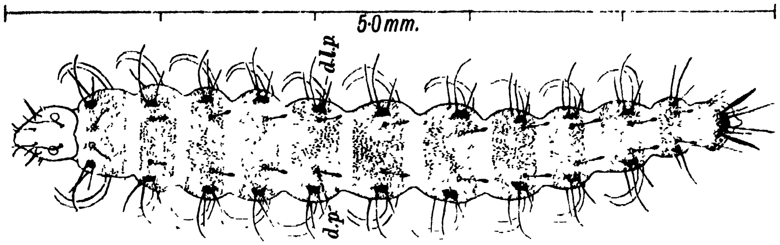

Fig. 1. Dorsal view of Larva.

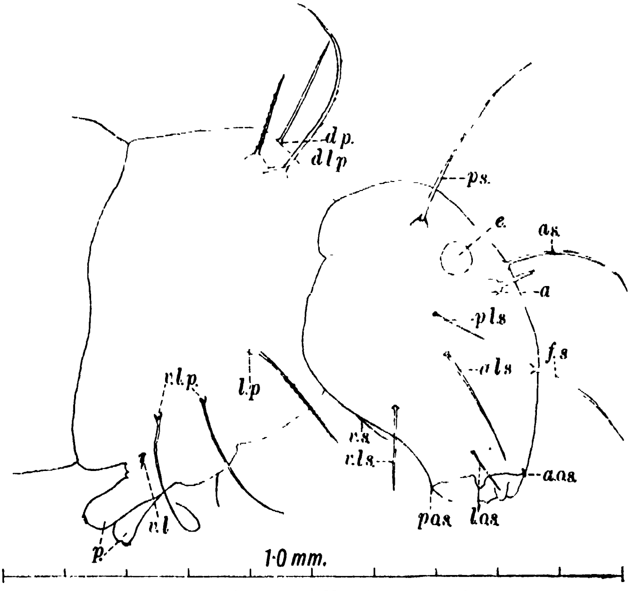

Fig. 2. Lateral view of Head and Prothorax.

forwards and outwards and is very slightly curved (*p.l.s.*). The longer seta (*a.l.s.*) arises antero-ventrally to the antenna, projects outwards and forwards and is also slightly curved. Ventro-laterally, midway between the mouth and the posterior border of the head, there is on each side a seta projecting downwards and outwards (*v.l.s.*). Slightly posterior to these, two small setae (*v.s.*) project downwards and forwards from the ventral surface. There are six short setae round the mouth; a pre-oral pair (*a.o.s.*) borne on the labrum, a lateral oral pair (*l.o.s.*), and a post-oral pair (*p.o.s.*) borne by the labium.

The Body consists of three thoracic and nine abdominal segments. Segments II to X are, except as regards size and the arrangement of the minute ventral setae which will be considered later, practically identical in external form. Each segment is broadest in the middle and decreases rather abruptly in diameter towards each end. Except for the inter-segmental regions and a narrow strip along the midventral region, the surface is covered with minute dark brown chitinous hooks. The dorsal ones point mainly backwards, the lateral ones mainly downwards.

Dorso-laterally each segment bears an oval papilla of dark brown chitin with an anterior and a posterior apex (*d.l.p.*). In the young larvae each of these papillae is at the summit of a large conical projection of the body wall, but in mature larvae the body wall becomes distended, chiefly by the increase in volume of the fat body, and these projections become practically obliterated. This is the condition shown in Text-fig. 1. Each papilla bears two setae, one on each apex. The anterior seta is strongly curved, projecting first upwards, outwards and slightly forwards and then bent strongly backwards. Under a low magnification it appears to be quite smooth, but with a high magnification it is found to have a few minute projections on its anterior surface. The posterior seta is straight, much shorter than the anterior one and bears numerous sharp projections pointing towards its apex (Text-fig. 2).

Dorsally each segment bears two small papillae of dark brown chitin (*d.p.*) from each of which a single straight and rather short seta projects upwards and backwards making an angle of about 45° with the surface of the segment. The apex of the seta is expanded into a small spear-shaped head which, in the living larva, always bears a small sphere of clear liquid. On each side of the segment is a small conical papilla (Text-fig. 2, *l.p.*) bearing a single seta. This seta is almost straight and has projections similar to those on the posterior dorso-lateral setae. Ventro-laterally the segment bears, on each side, two very small papillae situated close together (Text-fig. 2, *v.l.p.*) and each bearing a long and delicate curved seta. The basal parts of these setae are straight and project downwards and outwards, and are formed of denser chitin than their apical regions. Their apical two-thirds are bent strongly forwards and then upwards towards the body.

The chief peculiarity of the first body segment is its possession of a prominent ventral pseudopod (Text-fig. 2, *p.*). This is a large, freely movable appendage, more or less conical in form, arising by a broad base from the ventral region of the segment and having its distal end deeply cleft into right and left apices. Each apex is flattened and has a sucker-like appearance; each bears, close to its median border, a small circle of about six strongly chitinized simple hooks. The dorso-lateral papillae are smaller than those borne by the other segments, and the setae borne by the dorsal papillae have no spear-shaped head, are slightly curved and do not bear the drops of liquid mentioned above.

In segment XI each dorso-lateral papilla has only one apex and consequently bears only one seta which, from its size and shape, obviously corresponds to the anterior setae of the dorso-lateral papillae on other segments. There is only one ventro-lateral seta and this is short, only slightly curved, and projects outwards and backwards.

Segment XII differs considerably from any of the other segments. Postero-dorsally it bears a single large oval papilla, the long axis of which is transverse to the long axis of the body. This bears six small conical protuberances each bearing a single seta, all of which are practically straight. The setae are arranged in two transverse rows, four in the anterior row and two in the posterior row. The two median anterior ones project upwards and backwards, diverging somewhat from each other. The two lateral anterior setae project outwards and backwards, rising only very slightly from the horizontal; they are dorso-ventrally flattened. The two posterior setae project backwards and outwards. Two backwardly-projecting setae arise on each side from small papillae on the mid-lateral region of the segment, the more dorsal and posterior of the two setae being the longer.

Postero-ventrally the twelfth segment bears a prominent anal pseudopod on the surface of which is the anal opening (Text-fig. 3). The posterior edge of the pseudopod is produced into a median, backwardly projecting, conical lobe (*m.l.*) which bounds the anus (*an.*) posteriorly. On each side of the anus are two ventrally projecting conical lobes (*l.l.*). The anterior border of the pseudopod is in the form of a rounded semicircular rim which bears a number of prominent, dark brown, chitinous hooks with their apices pointing anteriorly. They are more or less arranged in two parallel semicircular series. Their shape is somewhat similar to that of a thorn on a briar stem; a curved hook arises from the centre of a basal bar which is firmly attached to the semicircular rim.

The minute ventral setae must be considered separately for thoracic and abdominal segments. The abdominal segments each bear six ventral setae, three on each side. In the first seven segments their arrangement is identical. Two are situated in the anterior half of the segment, one on each side of and close to the median line. In the posterior half of the segment there are two on each side rather far from the median line, the more laterally placed being also the more anteriorly placed of the two. In the eighth abdominal segment the anterior and posterior sets of setae are approximated so that they almost form a single transverse series near the middle of the segment. In the last abdominal segment the setae are more medianly situated than in the other segments so that they form two longitudinal rows, each of three setae, one on each side of and close to the median line (Text-fig. 3, *v.s.*).

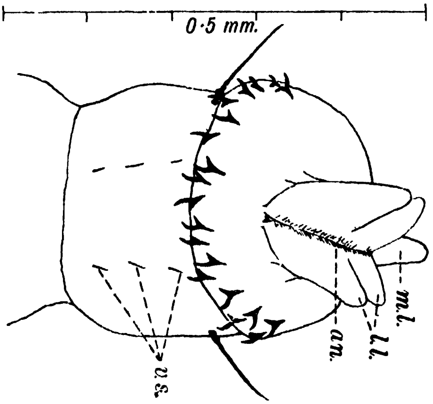

Fig. 3. Ventral view of last body segment of Larva.

Each thoracic segment bears on each side, near the lateral border of the ventral surface and in the anterior half of the segment, a single minute seta. In the case of the prothoracic segment these are situated on the anterior border of the pseudopod near its base. A short distance posterior to each of these single setae is a group of three very small and slender setae with their bases very close together. In the prothoracic segment they lie just lateral to the base of the pseudopod (Text-fig. 2, *v.l.*). In each group one of the setae is considerably shorter than the other two. These groups of three setae are undoubtedly the structures interpreted by Keilin (10) as vestigeal legs, which he believes to be present, with very few exceptions, in all Dipterous larvae. Their relation to the imaginal discs of the legs will be considered when dealing with these structures.

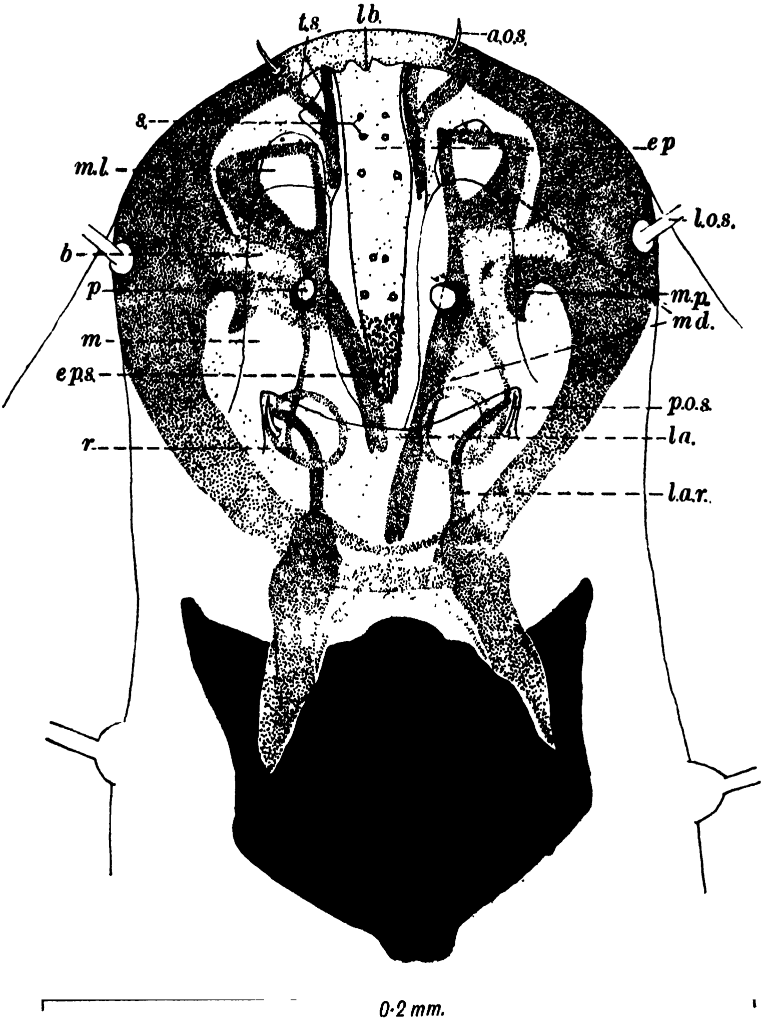

Fig. 4. Ventral view of Larval Mouth Parts. (From a specimen mounted in Canada Balsam.)

The Mouth (Text-fig. 4) is a narrow longitudinal slit on the narrow ventral end of the head. The Labrum (*lb.*) is a small plate overhanging the anterior end of the mouth and bearing the pre-oral setae (*a.o.s.*) on its dorsal surface. The Labium (*la.*) is a somewhat similar plate bordering the mouth posteriorly and bearing the two post-oral setae (*p.o.s.*) on its posterior or ventral surface. Its free edge is slightly concave, but does not show the conspicuous lobing of the true Chironomid larvae (16). A sagittal section of the head shows that the labium consists of an outer and an inner plate, both heavily chitinized, the free edge of the labium being the point of junction of the two plates. Posteriorly the outer plate is continuous with the ventral chitin of the head, the inner plate with the chitinous intima of the ventral wall of the pharynx. The labium is supported by two curved chitinous rods (*l.a.r.*) whose relations will be described later.

The Maxillae (*m.*) are very rudimentary. Each is a rounded swelling running along the lateral border of the mouth opening and continuous along its whole length with the lateral chitin of the head on one side and the chitin of the pharynx on the other. Its anterior end is differentiated as a rounded lobe (*ml.*) which appears to bear a few minute sense organs whose precise nature I have been unable to determine. Midway along its ventral surface it bears a prominent projection (*p.*) supported by a short hollow cylinder of firm chitin. On the anterior surface of this projection there are two small papillae each bearing an exceedingly minute seta which is probably sensory. The ventral projection is probably to be regarded as the maxillary Palp.

The Epipharynx (*ep.*) is a fold of the dorsal chitin of the mouth cavity, the anterior end of the fold being continuous with the inner surface of the labrum. The ventral surface of the fold lies along the mouth opening which it almost completely fills, its posterior end almost reaching the labium. It thus divides the anterior region of the mouth cavity into right and left halves. The posterior region of the epipharynx, both on its ventral surface and on the ventral regions of the lateral walls, is beset with numerous minute spines (*ep.s.*). The sides of the epipharynx, which are attached dorsally to the roof of the mouth, are thin and longitudinally wrinkled and so are capable of extension. Examination of a living larva shows that the epipharynx can be protruded from the mouth and performs sweeping movements backwards and forwards, its anterior end acting as a hinge. There is little doubt that by these movements the larva sweeps into its mouth the particles of decaying wood and bark upon which it feeds; the spines on its posterior end doubtless increase its efficiency in this respect. In serial sections I have indeed occasionally found such particles adhering to these spines. On the anterior region of its ventral surface the epipharynx bears five pairs of small sense organs (*s*). Sections of this region show that these are relatively deep but narrow cylindrical pits whose walls are formed of thin smooth chitin. It may reasonably be supposed that they are gustatory in function.

The Mandibles (*md.*) are two stout rods lying parallel to the long axis of the mouth opening through which no part of them is capable of protrusion. Their anterior ends are expanded and articulate with certain regions of the head skeleton which are described below. Their posterior ends are divided into three stout blunt teeth. They do not work against each other but against the inner plate of the labium, and they are only capable of motion through a small arc of a circle in the sagittal plane. They are not themselves capable of approximation to the labium which is normally slightly separated from their apices and consequently there has to be a mechanism, which is described below, whereby the labium is forced forwards against their apices. The strong retractor muscles of the mandibles arise from the postero-lateral regions of the head and converge in a fan-like manner to be inserted into a projection about half-way along the dorsal border of each mandible. The protractor mandibular muscles arise from the antero-lateral regions of the head capsule (anterior to the antennal bases) and converge towards the anterior articular end of each mandible. The tendon passes over the base of the mandible and is inserted into its ventral surface a short distance from its anterior end. The contraction of the retractor muscles draws the mandibular apices dorsally, *i.e.* into the mouth cavity. The protractor muscles have of course the reverse effect. The mandibles are capable of independent motion. Thus in Text-fig. 4 one mandible is in the retracted position while the other is protracted.

I am not aware of any previously described Orthorrhaphous larva whose mandibles move in the sagittal plane. In many (Becker, 1910) the mandibular apices work against the labium and against each other, but their direction of motion is oblique. In view of the fact that the mandibular apices in *Forcipomyia piceus* are incapable of protrusion through the mouth opening and therefore incapable of any prehensile or puncturing action, it is interesting to note that the larva of *Ceratopogon* (= *Forcipomyia*) *Laboulbene* is, according to Perris (21), occasionally predaceous and must therefore have rather differently constructed mouth parts. Perris does not, however, give a detailed description of the mouth parts, and it is not possible to state what the differences are.

The Head Skeleton (Text-figs. 4 and 5) consists of two regions, one being a special development of the chitin surrounding the mouth, the other situated near the centre of the head and being a special development of the chitinous intima of the pharynx. I am unable at present to give a full account of the structure of the latter region. The two parts are connected ventrally by chitinous rods.

The general shape of the pharyngeal skeleton is that of a V whose base is much thickened and is traversed by the pharyngeal cavity. The apices of the arms of the V lie just beneath the dorsal wall of the head slightly anterior to the antennal bases. Its base is connected by chitinous rods to the labium. Powerful muscles arise from the posterior dorso-lateral regions of the head capsule and are inserted into the posterior surfaces of the arms of the V near their bases. The contraction of these muscles pulls the pharyngeal skeleton and the attached labium upwards. The antagonistic muscles arise from the anterior dorso-lateral regions of the head capsule and are inserted into the anterior surfaces of the arms of the V, near their bases.

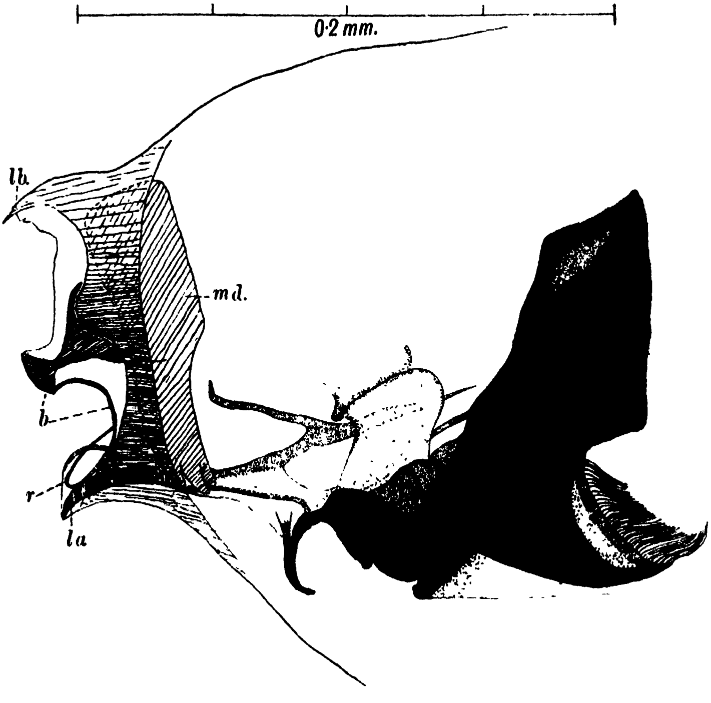

Fig. 5. Lateral view of Larval Mouth Parts and Tentorium. (Somewhat diagrammatic.) (From a potash cleared mount.)

Surrounding the mouth there is a chitinous ring which is very thick and hard except anteriorly. From the thin anterior region of the ring arise two triradiate sclerites (*t.s.*), one on each side. The two anterior arms of each of these sclerites are firmly attached to the ring, while the third arm projects horizontally backwards into the mouth. Slightly more posteriorly the ring gives off on each side a prominent mesially directed process (*mp.*) and the mandibles articulate between these and the posterior arms of the triradiate sclerites. Close to the base of each lateral process arises a long curved rod with a stout base which projects downwards and slightly mesially (*b.*). The main part of the rod is directed posteriorly at right angles to its base and ends posteriorly just in front of the labium, in a rather large ring (*r.*). The details of its course can be seen in Text-figs. 4 and 5. The curved rods supporting the labium have already been mentioned. Each passes through one of the rings described above, and the purpose of this arrangement is, I believe, as follows. When the labium is drawn upwards by the upward movement of the pharyngeal skeleton, the chitinous rings press against the posterior surfaces of the curved rods supporting the labium and these rods are curved in such a way that this pressure forces them, and the labium with them, forwards against the mandibular apices. This is, however, probably a secondary function only, and there are probably definite muscles for the movement of the labium towards the mandibular apices. The primary function of the chitinous rod and ring is probably to hold the labium in position against the pressure of the mandibles during mastication, and the curve of the rods bearing the ring probably allows a certain amount of play so that the labium is not held absolutely rigidly fixed.

EXTERNAL ANATOMY OF THE PUPA (Text-figs. 6 and 7).

The pupa emerges through a dorsal slit in the larval skin a short distance behind the head, but the posterior end of the pupal abdomen is never withdrawn from the larval skin. During the emergence of the pupa the prominent hooks on the anal pseudopod of the larva act as organs of fixation being firmly attached to the surface of the bark. They again perform this function at the time of emergence of the imago. The pupa drawn in Text-figs. 6 and 7 has been freed from the larval skin. The head, thoracic and abdominal regions are easily distinguished; it will be convenient to deal first with the second of these.

The Thorax is very convex dorsally and is produced posteriorly into a hollow, conical scutellar process (*s.p.*) which projects backwards over the first abdominal segment, and bears a pair of small rounded prominences, one on each side, about half-way along its dorsal surface. The scutellum of the imago occupies the anterior region of this hollow process, and in old pupae the scutellar bristles of the imago can be seen projecting into its posterior prolongation. A median ridge (*r.*), which is interrupted about half-way along its length by a small flattened area, extends from the anterior border about half-way along the dorsal surface of the thorax. This ridge is formed of two narrow strands of hard chitin, one along each side, the median part of the ridge being much thinner and less densely chitinized. The splitting of the pupal integument for the escape of the imago takes place along the thin apex of this ridge. There are eight slightly curved dorsal spines (*d.s.*) whose arrangement is sufficiently shown in Text-fig. 6. Antero-laterally the thorax bears on each side a short knobbed respiratory trumpet (*r.t.*).

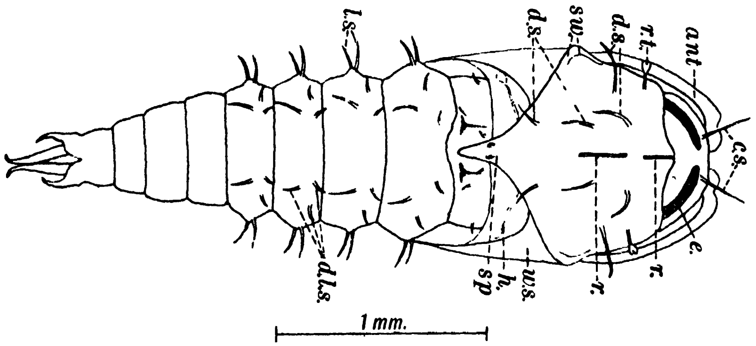

Fig. 6. Dorsal view of ♂ Pupa.

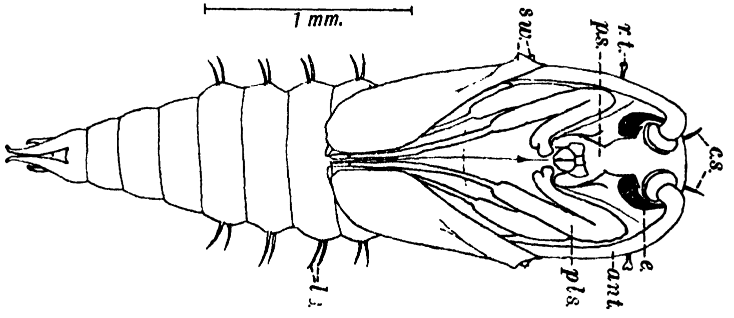

Fig. 7. Ventral view of ♂ Pupa.

The wing sheaths (*w.s.*) arise by a broad base from the lateral region of the thorax just anterior to the base of the scutellar process. Slightly dorsal to the base of each, the lateral wall of the thorax bears two rather prominent swellings, a dorsal and a ventral, situated close together (*sw.*). Beneath these swellings are developed the chitinous sclerites which take part in the articulation of the wing. The wing sheaths are directed posteriorly and curve round on to the ventral surface, being closely applied to the body wall. Their tips extend back to the anterior ventral region of the third abdominal segment. Their ventral edges lie very close together and between them are the distal ends of the leg sheaths. All but the coxal region of the prothoracic leg sheaths (*p.l.s.*) are clearly visible in a ventral view; only the tip of the coxal sheath can be seen. The disposition of the various parts is shown in Text-fig. 7. Their tips extend almost to the apex of the wing sheaths between whose ventral edges the tarsal region of the prothoracic leg sheaths lies. Only small parts of the meso- and metathoracic leg sheaths are visible, being for the most part covered by either the prothoracic leg sheaths or the wing sheaths. The tips of all of them can be seen between the ventral edges of the wing sheaths. There is no pupal sheath for the haltere though this can be seen clearly below the pupal integument (Text-fig. 6, *h.*). It base lies just below the antero-lateral border of the scutellar process. The shaft passes outwards and backwards and the head lies just above the dorsal border of the wing sheath.

The pupal *Head* region lies antero-ventrally to the thorax and is produced into a proboscis sheath (*p.s.*) which lies close against the antero-ventral region of the thorax, between the prothoracic femoral sheaths. The head bears two short and curved dorsal spines (*c.s.*). From its antero-lateral regions arise the antennal sheaths (*ant.*) which bend back along the lateral borders of the head and thorax, their distal ends being covered by the wing sheaths. In male pupae the base of the sheath is considerably more swollen than in female pupae, corresponding to the greater development of Johnston's organ in the male imagines. The eyes of the imago (*e.*) can be seen beneath the pupal integument occupying the sides of the head just posterior to the bases of the antennae. In old pupae they become very prominent owing to their dense black colour.

Shortly before the emergence of the imagine the sheaths of the various appendages separate slightly from each other and from the surface of the pupa, thus facilitating the withdrawal of the appendages of the imagine.

The pupal *Abdomen* is conical in shape, narrowing rapidly posteriorly, and consists of nine clearly defined segments. The last three, and the posterior two-thirds of the sixth never become freed from the larval skin, and it is probably owing to this that they possess none of the prominent spines which are a conspicuous feature of the first five abdominal segments. These abdominal spines are of the same type as the thoracic ones and are arranged in a dorso-lateral and a lateral series on each side of the body. On segments II to V they are identical in arrangement; each segment bears on each side a pair of lateral spines at about the mid-point of its lateral surface, the two being close together, one slightly dorsal to the other (*l.s.*); dorso-laterally are three spines on each side whose arrangement is shown in Text-fig. 6 (*dl.s.*); the posterior spine of each group is the largest. The lateral regions of the first abdominal segment being covered by the wing sheaths no lateral spines are developed. Dorsolateral spines are present but are smaller than those on the other segments and somewhat differently arranged (Text-fig. 6). In male pupae the last abdominal segment is produced backwards into four processes, two larger ventral and two smaller dorsal ones (Text-figs. 6 and 7). In the female pupae only the ventral processes are present. The pupal chitin bears numerous small blunt papillae, considerably smaller than the hooks borne by the larval chitin. The pupal spines are hollow protuberances of the pupal chitin and are not connected to the pupal chitin by a joint. Each lies over a group of somewhat enlarged hypoderm cells each of which sends a protoplasmic prolongation into the cavity of the spine. They thus agree in all respects with Comstock's definition of a "spine" (5) and differ very greatly from the larval "setae." They have an irregularly ringed appearance as though made up of several joints. Under a high magnification this appearance is seen to be due to a number of minute projections on the seta arranged in an irregularly annular manner.

The Respiratory Trumpets consist of a short hollow cylindrical shaft and a distal knob. A trachea runs up the centre of the shaft and opens to the exterior by a small aperture at the apex of the knob. In the shaft the trachea is surrounded by a thin cellular layer which becomes many cells thick in the knob whose cavity it almost fills. The trachea does not show any spiral thickening but its intima is raised up into numerous spines. These are particularly prominent in the part of the trachea lying in the knob and probably act as an occluding apparatus.

INTERNAL ANATOMY OF THE LARVA.

The Gut is a cylindrical and practically straight tube, consisting of fore-gut, mid-gut and hind-gut. The Pharynx, which is the anterior region of the fore-gut, extends through the anterior half of the head and opens ventrally at the mouth. I am at present unable to give any account of its structure, but I believe that the complicated sclerites formed from the chitinous intima have a triturating and a straining function.

The Oesophagus is a straight tube of uniform diameter proceeding directly backwards from the posterior end of the pharynx. It traverses the ganglionic mass in the first thoracic segment and ends in the Cardiac Valve in the second thoracic segment. Internally it is lined by a chitinous intima which is produced into irregular folds. The epithelium is thin and is only distinct (in sections) in the bases of the folds of the intima. The muscular coat is several fibres in thickness and consists exclusively of circular muscles.

Text-fig. 8 is a slightly diagrammatic representation of a sagittal section through the cardiac valve. The oesophagus is continued unchanged down the centre of the valve, at the posterior end of which its epithelium (*e.* and *e'.*) and chitinous intima (*i.* and *i'.*) are reflected outwards and forwards to the anterior end of the valve. Here (*a.*) the reflected layer of epithelium turns posteriorly to become continuous with the epithelium of the cardiac region of the mid-gut (*c.*). The muscular layer of the oesophagus (*m.*) is continued down the valve to its posterior end but is not reflected forwards again as is the epithelium. The reflected chitinous intima is continuous over the whole extent of the reflected epithelium from which, however, it is widely separated except at the point where the reflected epithelium becomes continuous with the cardiac epithelium; at this point the chitinous intima ends. Slender ligaments (*l.*) pass from the wall of the oesophagus to the point where the reflected epithelium turns posteriorly.

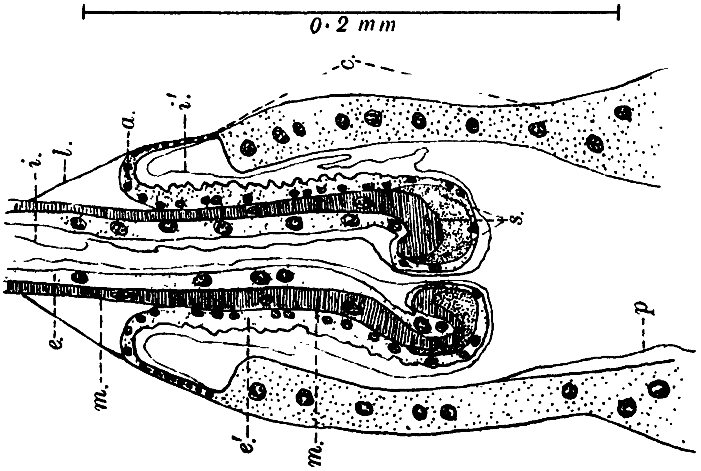

Fig. 8. Sagittal Section of the Larval Cardiac Valve. (Slightly diagrammatic.)

In *Chironomus* and *Simulia* according to Miall and Hammond (18) and in *Anopheles* according to Imms (8) there is a fairly extensive blood sinus between the two layers of the cardiac valve. In *Forcipomyia* the two layers are in complete apposition except posteriorly; the posterior end of the valve is somewhat swollen and between the two layers there is a space (*s.*) which I regard as a blood sinus. Compared with the corresponding sinus in the three types mentioned above it is greatly reduced in size, and I am unable to find any communication between it and the general body cavity of the larva.

The reflected epithelium, just in front of its point of junction with the cardiac epithelium, is composed of exceedingly small cells so that the point of junction of the two epithelia is very distinct. A study of late larval stages shows that these small cells are imaginal cells which form the posterior region, and possibly the whole of the imaginal oesophageal epithelium. A ring of imaginal cells in this position is of common and possibly universal occurrence in Dipterous larvae. It is recorded for instance in *Musca* by Kowalevsky (12) and van Rees (24) and in *Chironomus*, *Anthomyia*, *Stratiomys*, *Tanypus* and *Gastrophilus* by Vaney (23). A similar ring occurs in many other holometabolous larvae, *e.g.* in *Formica rufa* according to Pérez (20), and in *Hyponomeuta padella* according to Mme Hufnagel (7).

The cells of the cardiac epithelium are essentially similar to those of the mid-gut epithelium generally except that they never give off masses of secretion (see below) and are non-vacuolated except in late larval stages. There are no muscle fibres in the wall of the cardia.

Posteriorly the cardia becomes continuous with the stomach which is a straight cylindrical tube extending back to the anterior region of the eighth body segment where it joins the hind-gut. It is lined internally throughout its length by a cylindrical Peretrophic Membrane (Text-fig. 10, *p.*). Its wall (Text-fig. 10) consists of a large celled epithelium (*e.*), an inner layer of circular muscles one fibre thick (*c.m.*), an outer layer of isolated longitudinal fibres (*l.m.*) and an external sheathing membrane. The peretrophic membrane is a delicate tube of uniform diameter throughout. In the cardia (Fig. 8) it lies close against the surface of the epithelium from which it can hardly be distinguished unless it is broken away in cutting the sections (*p.*). The diameter of the mid-gut cavity is in most places greater than that of the cardiac cavity, and as the diameter of the peretrophic membrane is constant throughout and equal to the diameter of the cardia, it is in most places widely separated from the epithelium of the mid-gut. For this reason I am inclined to agree with the generally accepted view that the peretrophic membrane is produced by the cells of the cardia. According to Imms (8) this is probably its mode of origin in *Anopheles* but in *Chironomus* it appears, according to Miall and Hammond (18), to be derived from the epithelium of the stomach "either by secretion or conversion."

The epithelium of the stomach consists of large, and for the most part columnar cells with prominent nuclei. Their bases are usually conspicuously vacuolated. Between the internal faces of the cells and the peritrophic membrane, in the middle two-thirds of the stomach, there is usually a granular mass with a slight affinity for plasma stains such as Lichtgrün and Eosin (Fig. 10, *g.s.*). That this mass is secreted by the gut cells may be very clearly seen in almost every transverse section of the region where the secretion occurs. The inner ends of the cells are normally somewhat conical in shape and are formed of densely staining non-vacuolated protoplasm. Certain of them may be seen to have swollen and conspicuously protruding apices, while in others these apices have burst and their contents mingle with the mass of secretion lying between the epithelium and the peretrophic membrane. Gehuchten (6) first drew attention to this peculiar method of secretion in the larva of *Ptychoptera contaminata*, but it has since been found to occur in many insects.

Slightly posterior to the cardia the epithelium of the stomach shows a ventral thickening. The degree of development of this ventral thickening varies considerably in different larvae, but I have not been able definitely to correlate its degree of development with the degree of maturity of the larva. The cells of the thickening usually exhibit considerable vacuolation, the individual vacuoles being small. In an example which had a very prominent ventral thickening, examination with an oil immersion lens showed what appeared to be minute branching ducts opening into the gut lumen through the peretrophic membrane which is here closely applied to the surface of the epithelium. It seems probable that the ventral thickening is of a secretory nature. The epithelium of the anterior and posterior sixths of the stomach does not form globules of secretion. The malpighian tubes open into the posterior end of the mid-gut and not into the anterior end of the hind-gut. The same exceptional condition occurs in *Ptychoptera* according to Gehuchten (6) and in the *Meloidae* according to Beauregard (1). The relation of the malpighian tubes to the gut will be considered later. The posterior end of the mid-gut epithelium is continued for a short distance into the anterior end of the hind-gut forming the Pyloric Valve. Small imaginal cells (*i.c.* Fig. 10) occur scattered irregularly along the mid-gut between the bases of the epithelial cells. They are usually placed singly, but occasionally two or three occur together in a small clump.

The hind-gut extends in a practically straight line from the anterior region of the eighth body segment to the anus and is divisible into three regions, an Ileum extending back to the middle of segment X, followed by a Colon extending back nearly to the middle of segment XII, and terminating in a short Rectum which opens ventrally by the anus. The epithelium of the anterior end of the ileum is folded inwards to become continuous posteriorly with the mid-gut epithelium which is continued into the cavity of the ileum. This inner layer of the epithelium of the ileum consists of imaginal cells which proliferate at the time of histogenesis to form the imaginal hind-gut. The wall of the ileum consists of a well-marked epithelium (except in the extreme anterior region where it is very thin) and two layers of muscles, a thin inner layer of isolated longitudinal fibres and a continuous and rather thick outer layer of circular fibres. In the extreme anterior end the intima is raised into small papillae. In the remainder of the ileum the intima, and with it the epithelium, is raised up into four folds, two large lateral, a small ventral, and a small dorsal. The colon differs from the ileum in having no longitudinal muscles and its circular muscles are in the form of separate thick bands placed at small intervals round the gut. The lateral folds of the intima and epithelium are unchanged, but there are two small dorsal and two small ventral folds. The chitinous intima, particularly on the lateral folds, is produced into blunt spines. The Rectum is a terminal dilatation of the colon. Its epithelium and intima are folded in a complicated manner and the latter is produced into prominent spines pointing posteriorly. No longitudinal muscles are present and the circular muscles are similar to those of the ileum.

The Salivary Glands (Text-fig. 9) are of a peculiar type. Their anterior ends lie dorsal to the first thoracic ganglion, near the median line. From here they extend postero-dorsally as lobed strap-like bodies to the anterior region of the cardiac division of the stomach when they narrow considerably, turn backwards dorso-lateral to the gut and extend back into the first abdominal segment. Their anterior ends are irregularly circular in outline and composed of large cells surrounding a central duct which is a direct continuation of the salivary duct. The middle region of the gland is only one cell thick and is irregularly lobed. The narrow dorsal region consists of only two cells in any cross-section, one dorsal to the other. The cell limits are everywhere very indistinct owing to the fusion of the cells. The central duct extends a short distance into the gland from its anterior end, but beyond its termination there is no central cavity about which the cells are arranged. The secretion is collected from the cells and poured into the main duct by a series of minute ducts. A few of these (*d.*1) appear, at all events in parts of their course, to be intercellular, lying between two adjacent cells. It is difficult to be certain on this point owing to the impossibility of defining the cell limits. The majority of the collecting ducts are, however, undoubtedly intracellular and appear in cross-section as minute, circular vacuoles in the cell protoplasm, lined by an exceedingly delicate, possibly chitinous, membrane (*d.*2). The cells of the gland are large and much vacuolated. They contain a large spherical nucleus provided with a prominent rounded nucleolus and a considerable amount of chromatin.

Each gland gives off a short duct anteriorly which passes forwards to one side of the connective joining the sub-oesophageal and first ventral ganglia, joining with its fellow of the opposite side immediately in front of this connective to form a median salivary duct which runs forwards to open into the posterior wall of the mouth. The salivary duct consists of a thin non-glandular cellular sheath and a chitinous lining which shows spiral thickenings.

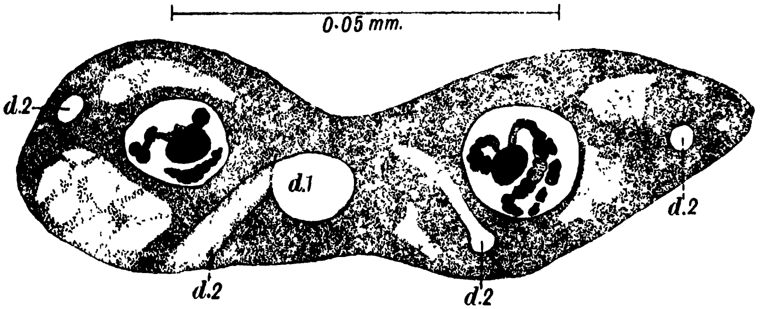

Fig. 9. Transverse Section of a portion of the Salivary Gland in the region of the Cardiac Valve.

So far as I am aware no previous case has been recorded among Dipterous larvae of salivary glands which discharge their secretion by means of intracellular ducts. In *Gli Insetti* (I, 516) Berlese (3) gives a figure of the labial salivary glands of *Mytilaspis*, the cells of which discharge by means of intracellular ducts, but this is the only instance of such glands which I have seen. In the vast majority of insects the salivary glands are hollow vesicles or tubes with a lining layer of secretory epithelial cells discharging into a central lumen, which is continuous anteriorly, directly or indirectly, with the salivary duct.

The Malpighian Tubes are seven in number, four of them being anteriorly directed and three posteriorly. The posterior end of the mid-gut shows a slight dorsal thickening of the epithelium and from each side of this arises a main malpighian tube trunk. The main trunk is short and from its apex arise three dorsal malpighian tubes, two anterior and one posterior.

The two anterior malpighian tubes of each side extend forwards into the posterior region of the third thoracic segment; along their whole extent they are close together, and in places are quite contiguous for a short distance and appear to be united. At intervals they come into close relation with the gut wall and are apparently attached to its sheathing membrane. The two tubes of one side never come into this close relation with the gut at the same point. The anterior tubes show undulations, but no convolutions or sharp bends. The two posterior dorsal malpighian tubes are directed straight backwards and extend to about the posterior limit of the eleventh body segment. They lie dorso-lateral to the gut and do not come into close connection with it at any point. From the ventral region of the posterior end of the mid-gut arises a single ventral posterior malpighian tube. It runs back in the mid-ventral line below the gut as far as the tenth segment. It then bends across to the right, turns forwards and then upwards until it is close to the right posterior dorsal malpighian tube, when it turns backwards parallel to the latter, the two ending at about the same level.

The malpighian tubes are all similar in structure. Each consists of a row of large, approximately cylindrical cells placed end to end. There is a continuous intracellular lumen traversing the whole series. The nuclei are large and rich in chromatin. The shape of the lumen varies somewhat, being sometimes circular, sometimes rayed in cross-section.

The Central Nervous System consists of supra- and sub-oesophageal ganglia, united by oesophageal connectives, and ten ventral ganglia. The supra-oesophageal ganglion is a bilobed mass, the two lobes being united anteriorly by a broad transverse commissure. The posterior ends of the two lobes are separate. Antero-laterally each lobe gives origin to a very short oesophageal commissure which passes forwards and downwards to unite below the oesophagus, with the posterior end of the sub-oesophageal ganglion. Thus the sub-oesophageal ganglion is more anteriorly situated than the supra-oesophageal, and extends as far forwards as the anterior limit of the first thoracic segment. The oesophageal commissures are broad and very short and are hardly distinguishable from the general mass of the brain.

The ventral ganglia are distributed as follows:

1st Ganglion.	Posterior limit of first body segment.
2nd ,,	Middle of second segment.
3rd ,,	Anterior third of third segment.
4th ,,	Anterior limit of fourth segment.
5th ,,	,, ,, fifth ,,

6th	Ganglion.	Anterior limit of sixth segment.
7th	,,	,, ,, seventh ,,
8th	,,	,, ,, eighth ,,
9th	,,	Between segments eight and nine.
10th	,,	Anterior third of ninth segment.

Between the 1st and 2nd ganglia there are two widely separated connectives. Between ganglia 2 and 3 the connectives are also double but closely approximated; the remaining connectives are apparently single. The ganglia do not vary greatly in size; the seventh is, however, the smallest and the tenth the largest. The tenth ganglion gives off five large posteriorly directed nerves. All ganglia consist of a fibrous tract surrounded by nerve cells which are mainly concentrated ventral to the fibrous tract.

The sub-oesophageal ganglion gives off three nerves on each side from its anterior end, the two dorsal nerves being the largest. Each dorsal nerve goes to a pharyngeal ganglion situated close to the lateral wall of the pharynx a short distance behind the mouth (Text-fig. 13 A, *p.g.*). On each side one of the ventral nerves goes to a small ganglion situated on the Labial Imaginal Disc (Text-fig. 13 C, *l.g.*). The other two ventral nerves appear to be distributed to the ventral hypoderm of the head and some of the cephalic muscles. The above two pairs of ganglia, and a median ganglion, which is described in connection with Weismann's Ring, belong to the stomato-gastric system.

The Dorsal Vessel is a long tube situated slightly dorsal to the gut and extending from the anterior region of the twelfth body segment to the posterior region of the head. In the twelfth segment an arched membrane separates off a dorsal pericardial chamber from the remainder of the segment, the chamber being narrow anteriorly and widening out posteriorly. The posterior end of the dorsal vessel projects backwards for some distance into the pericardial chamber, in the extreme anterior end of which the dorsal and ventral walls of the vessel fuse with the dorsal and ventral walls of the chamber. Thus a cross-section of this region shows three cavities; a central one which is oblong in shape with its long axis vertical, and a lateral one on each side of it and somewhat arc-shaped. The central cavity is that of the dorsal vessel, the lateral cavities are the anterior regions of the pericardial chamber. As it passes backwards into the pericardial chamber, the diameter of the vessel diminishes rapidly and its narrow posterior end is quite free from the pericardial walls and opens into the chamber. I have been unable to find any ostia in the dorsal vessel and the method of "blood" propulsion appears to me to be as follows. If one supposes the pericardial chamber and the dorsal vessel to contract simultaneously, the blood in the chamber will be forced into the anterior regions of the chamber which lie one on each side of the posterior prolongation of the vessel. The distension of these lateral chambers will tend to compress and close the posterior end of the vessel. Contraction of the vessel will now force its contained blood forwards into the head. On relaxation of the vessel and the pericardial chamber the low pressure in the former will tend to draw blood into it from the latter. As the posterior end of the vessel projects freely into the pericardial chamber and has a very small opening, I think it improbable that blood can be actively forced from the chamber into the vessel.

Posteriorly the dorsal vessel is wide and circular in cross-section, but it narrows considerably and becomes dorso-ventrally flattened as it passes forwards. In the first thoracic segment it traverses the ganglionic mass, lying immediately dorsal to the oesophagus. Delicate strands, which appear to contain no nervous elements, pass from the sheathing membrane of the oesophageal commissures and fuse with the wall of the vessel. In front of the ganglionic mass its shape in cross-section is that of a rather narrow isosceles triangle with its base lying on the dorsal wall of the oesophagus. It retains this shape to a point slightly posterior to the pharynx where it opens freely into the head cavity. The part of the vessel lying in the head and thorax is not provided with pericardial cells and may perhaps be distinguished as the Aorta, though there is no true morphological distinction between it and the remainder of the vessel. The wall of the heart is composed of circular striated muscle fibres whose nuclei occur in the lateral walls in which they form conspicuous bulges (Text-fig. 10, *h.nu.*).

The Pericardial Cells (Text-fig. 10) occur in pairs, one on each side of the dorsal vessel in segments IV to XI. The pairs of cells are separated by considerable intervals and lie upon the dorsal parts of the alar muscles (*a.m.*) with which their protoplasm appears to be continuous, as is the case in the Blow-fly larva according to Lowne (14). There is thus a pericardial cell in connection with each alar muscle, and this arrangement is very common and possibly universal in Dipterous larvae. Pantel (19) in *Thrixion Halidayanum* and Imms (9) in *Anopheles maculipennis* find that on approaching the dorsal vessel the alar muscles split into a dorsal and a ventral layer of fibres, the pericardial cells lying between the two layers. I have myself found a precisely similar arrangement in the larva of *Chlorops taeniopus*. In *Forcipomyia*, however, this splitting does not occur and the alar muscles are inserted into the ventral wall of the heart only. I have been unable to trace the alar muscles to their origin which is probably in the dorso-lateral body wall. They are probably striped muscles, but none of my preparations shows this clearly. It is possible that at the time when the larvae were fixed the alar muscles had commenced to degenerate in preparation for histolysis, and their staining properties had consequently been altered.

Each pericardial cell contains three or four nuclei (*p.nu.*), but there is no corresponding indication of cell division. The nuclei are rather small and contain a conspicuous nucleolus. Towards the end of larval life (which is the stage shown in Fig. 10) the pericardial cells become much vacuolated, and some of the larger vacuoles may be seen to contain a homogeneous body of very regular form (*s.*). Kowalevsky (12, 13) has proved that the pericardial cells have an excretory function, and it seems probable that the homogeneous lightly staining inclusions are composed of waste products extracted from the blood.

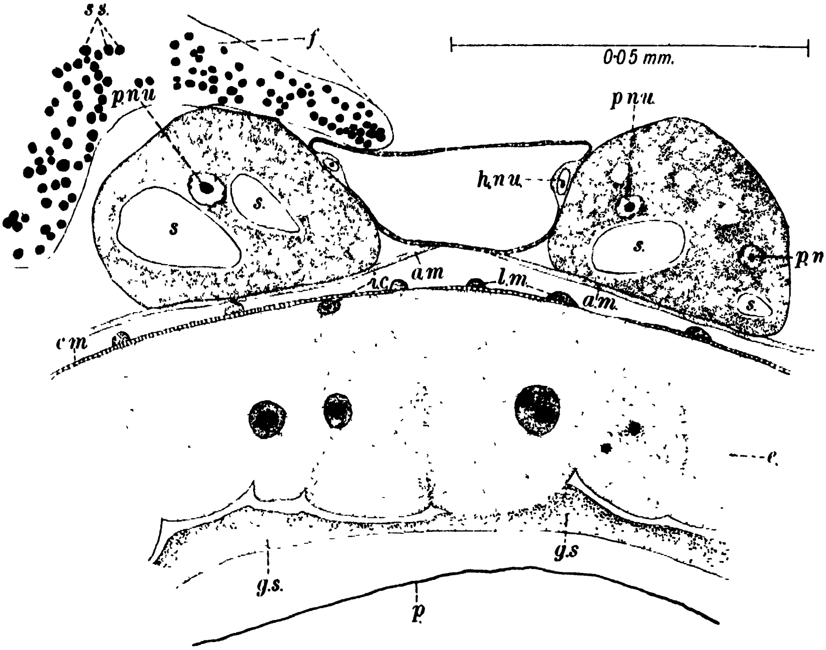

Fig. 10. Transverse Section through the dorsal wall of the Gut, the Dorsal Vessel and the Pericardial Cells. Posterior Region of Mid-gut.

In many, if not all, *Muscid* larvae there are ventral "pericardial" cells usually in the form of a transverse loop of cells which passes below the gut, its ends being in intimate relation with the anterior ends of the salivary glands. Such a strand is absent in *Forcipomyia* and has not, so far as I am aware, been recorded in any *Orthorrhaphous* larva. There are, however, certain cells (Text-fig. 13 A, *c.c.*) which will be mentioned in connection with the labial imaginal disc, which may possibly be of the same nature.

Considering the intimate relation between the dorsal pericardial cells and the alar muscles it is not, I think, improbable that the former are muscle cells of which the alar muscles are part, and that they have become considerably hypertrophied and specialized for an excretory function. Very careful embryological work is of course necessary to settle this point. The following passage from Lowne (14) (p. 343) dealing with the fate of the pericardial cells during the metamorphosis of the Blow-fly larva does, however, give some support to this theory. He says: "The persistent pericardial cells are probably the elements from which the alar muscles of the pericardium of the imago are developed, as I find them embedded in the substance of their fibres."

Weismann's Ring is here in the form of a straight solid cellular rod. Each lobe of the dorsal ganglion gives off from the mesial face of its posterior end a small mass of nervous tissue from the base of which a nerve passes vertically downwards close to the lateral wall of the dorsal vessel, the two nerves uniting to form a small ganglion lying between the ventral wall of the heart and the dorsal wall of the oesophagus, slightly posterior to the oesophageal commissures. I consider this to be the median ganglion of Lowne (14). The two mesially directed nervous masses mentioned above fuse together in the median line between the posterior lobes of the dorsal ganglion, and from their point of junction Weismann's "Ring" proceeds backwards as a thin solid rod of small cells. This rod extends back as far as the cardiac region of the gut, lying, except its anterior end, on the dorsal wall of the heart. In transverse section its anterior region shows more or less distinct indications of consisting of right and left halves. It gives off no strand to the wall of the cardia as does the homologous structure in *Cyclorrhaphous* larvae, from which it also differs in being a solid rod instead of a ring surrounding the dorsal vessel. It is composed of very small cells similar to the cortical cells of the various ganglia. The cell limits are indistinct.

The Fat Body (Figs. 10, 11 and 12, *f.*) varies in extent according to the age of the larva, being most extensive in mature larvae. In all stages it is entirely absent from the head. In a larva killed about the middle of January its general distribution is that of a rather thin and almost continuous sub-hypodermal layer giving off lobes between the various organs. In the abdominal segments it is mainly concentrated into two ventro-lateral masses.

The fat body cells are large and the cell limits are for the most part distinct. The cells contain very numerous small spheres (Fig. 10, *s.s.*) of a homogeneous substance, which stains dark blue with Heidenhain's Iron Haematoxylin. These are probably proteid storage products resulting from the digestive metabolism. In sections stained with Heidenhain's Iron Haematoxylin the nucleus is usually completely masked by the blue spheres; Erlich's haematoxylin, however, does not stain the spheres and the nucleus may easily be examined. The nuclei are spherical and rather small and contain a prominent nucleolus; their chromatin is mainly peripheral in position. The protoplasm is difficult to study on account of its poor affinity for stains. It appears to be in the form of trabeculae surrounding numerous vacuoles.

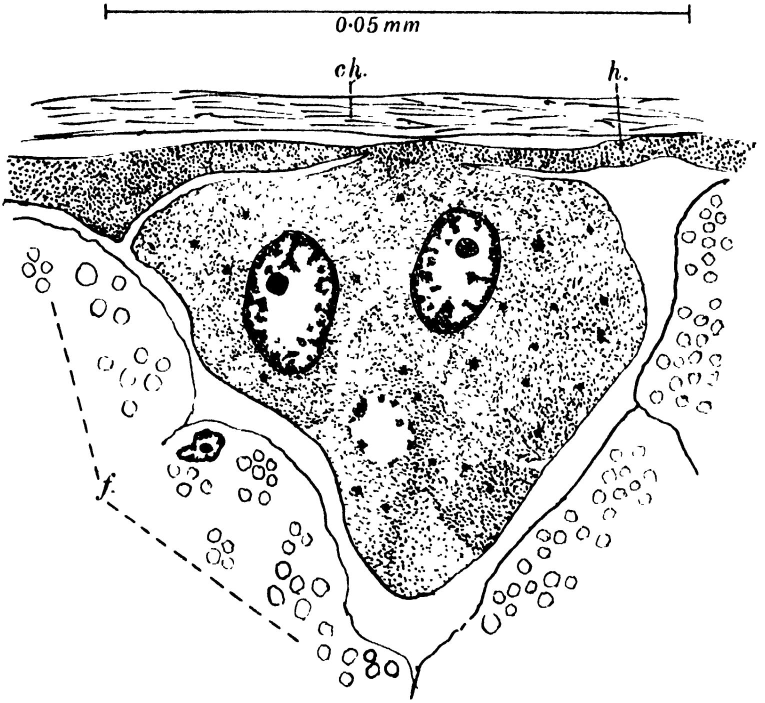

Fig. 11. Transverse Section of Larval Oenocytes. *ch.*, chitin; *h.*, hypoderm.

Large Oenocytes (Fig. 11) occur in groups in segments IV to XI. According to Mme Hufnagel(7) there are in *Hyponomeuta padella* two sets of Oenocytes, larval and imaginal. The former degenerate during metamorphosis, and the latter develop during the last larval stadium. Although I have only examined the last larval stage of *Forcipomyia*, I believe that here also there are larval and imaginal oenocytes as I have found what appeared to be the last stages of degeneration of the larval oenocytes close to the positions occupied by the oenocytes to be described. If this view is correct the bodies discussed below are the imaginal oenocytes.

On each side of segments IV to XI inclusive there is a mass of four large cells close to, and connected with, the hypoderm, in the ventro-lateral region of the segment (Fig. 11). During the pupal period they become separated from each other and from the hypoderm and take up various positions in the body. Their protoplasm is densely staining (with Eosin or Lichtgrün), non-vacuolated and rather homogeneous in appearance, but has a few small granules scattered through it. The nucleus is, except for its larger size, very similar to a hypoderm nucleus. It contains a large nucleolus and a considerable amount of chromatin mainly aggregated round the periphery of the nucleus. It seems fairly obvious that the oenocytes are special developments of certain hypoderm cells. This is shown in Fig. 11.

A few *Small Oenocytes* are present scattered through the body. They are not, however, nearly so numerous as they are in *Chironomus* according to Miall and Hammond (18) and in *Anopheles* according to Imms (9).

The Tracheal System is entirely closed. Perris (21) describes the larva of *Ceratopogon* (= *Forcipomyia*) *Laboulbeni* as having nine pairs of spiracles, one on each side of the prothorax and first eight abdominal segments. Malloch (16) says in his synopsis of the characters of the larvae of the Certaopogonidae "...in the terrestrial forms I am unable to find lateral abdominal spiracles." He does not mention any thoracic spiracles. After a careful examination of living larvae, potash cleared mounts and serial sections, I am convinced that stigmata are entirely absent in the larva of *Forcipomyia piceus*.

Tracheae are, however, present and ramify fairly extensively through the body. There are two main longitudinal trunks situated one on each side dorso-lateral to the gut and close to it, and united by a series of dorsal commissural tracheae. I have not been able to follow out the details of the tracheal branches as the larva is a very unfavourable one for the study of these by transparence. The chitinous intima of the tracheal trunks has no spiral thickening. The presence of a well-developed tracheal system in the absence of either stigmata or tracheal gills is peculiar but by no means unique, many similar instances being supplied, for example, by the parasitic *Hymenoptera* (Seurat (22)). The larva lives in a moist atmosphere and gaseous exchanges probably take place through the thin chitin of the body wall, the body fluid acting as an oxygen carrier. It is usually denied that the body fluid of insects has any respiratory function except in those *Chronomid* larvae whose body fluid contains haemoglobin. The point has, however, never been definitely proved and it is difficult to conceive how respiration can take place in the larva of *Forcipomyia* unless the body fluid has a respiratory function.

In the first thoracic segment there is on each side a very peculiar tracheal branch (Text-fig. 12) going to the hypoderm at a point slightly postero-lateral to the dorsal seta of its side. Before reaching the hypoderm it swells out into a conical ampulla whose base would lie against the hypoderm (*h.*) but has been pushed into the cavity of the ampulla. In this pushed-in base lies a peculiar formation of the hypodermis which consists almost exclusively of a relatively enormous hypoderm nucleus (*h.nu.*). The tracheal trunk which ends in the ampulla has no cellular sheath. This structure is probably to be interpreted as a vestigeal stigma; these have been recorded in *Anopheles maculipennis* by Imms (8) and in *Hypoderma bovis* by Carpenter and Pollard (4). There is no trace of any opening through the chitin (*ch.*).

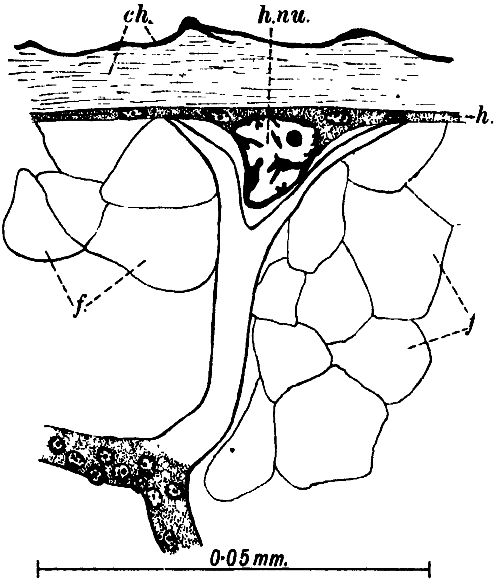

Fig. 12. Section through Vestigeal Stigmatic Trunk in Larval Prothorax.

Integument and Hypodermis. Except on the ventral non-hook bearing region, the body chitin consists of two layers, a thin and dark coloured outer layer and a thicker and lighter coloured inner layer (Text-fig. 12, *ch.*).

The small integumentary hooks consist exclusively of the dark outer layer, and it is this layer which is absent from the ventral body region. The hypoderm (*h.*) is a thin cellular layer, one cell thick, the cell limits being indistinguishable. The oval nuclei form small bulges in the layer. They contain a small spherical nucleolus and their chromatin is mainly peripherally situated.

The dorso-lateral and dorsal setae of the body are lodged over special hypoderm cells which are enormously hypertrophied. Their nuclei differ from those of ordinary hypoderm cells in having the chromatin scattered throughout the nucleus instead of concentrated round its periphery; they contain a large nucleolus. The dorso-lateral setae have a central cavity extending almost to their tips, and this cavity is occupied by an axial protoplasmic filament given off from the basal cell. In the dorsal setae the central cavity extends right to the tip of the seta in the spear-shaped head of which it becomes somewhat enlarged, the whole cavity being occupied by a protoplasmic continuation of the basal cell. It is obviously this cell which secretes the drop of liquid found at the apex of each dorsal seta in the living larva. The large cephalic setae resemble the dorso-lateral body setae. The remaining setae of the body are solid and are seated upon small thickenings of the hypoderm composed of several slightly hypertrophied cells instead of one greatly hypertrophied cell. The dorsal, dorso-lateral and lateral setae of the body are attached to the body wall by distinct articulations, but this does not appear to be the case for the ventro-lateral setae or the minute ventral setae.

The Cephalic Imaginal Discs consist of the rudiments of the imaginal antennae, mouth parts and eyes. Text-fig. 13 A, B and C represents the *Labial* imaginal disc sac as seen in the 30th, 32nd and 35th of a series of transverse sections. The imaginal sac arises in two lateral halves from the ventral hypoderm of the head and projects forwards below the pharynx. In its posterior region each lateral half is a hollow, approximately cylindrical body with its median wall much thickened (C). The salivary duct (*s.d.*) lies between the two halves. There is a small Labial Ganglion (*l.g.*) on the dorsal surface of each half. Anteriorly the two halves unite to form a single hollow sac which is U-shaped in cross-section and has its dorsal wall much thickened (B). The salivary duct lies in the concavity of the U. As it approaches the mouth the sac decreases in diameter (A) and finally ends blindly within the posterior region of the larval labium. Slightly behind its anterior end it receives a nerve from each of the two Pharyngeal ganglia (*p.g.*). At this point there is a transverse row of four large cells (*c.c.*) lying between the labial disc and the ventral wall of the pharynx. These have some resemblance to the dorsal pericardial cells (they do not resemble those shown in Text-fig. 10 which is from a section of a very late larval stage after the commencement of histolysis; Text-fig. 13 is from a much earlier stage), and it is possible that they represent the ventral pericardial cells of the *Muscidae* though these occupy an entirely different position. Feeding experiments with colouring matters similar to those used by Kowalevsky (12) in his experiments on *Muscid* larvae, can alone determine whether these cells are or are not ventral pericardial cells.

It is evident that the Labial disc as described and figured here is an immature stage, and really consists only of the imaginal sacs, the imaginal discs having not yet formed except as mere thickenings of the wall of the sacs. I am not able at present to give an account of the later development.

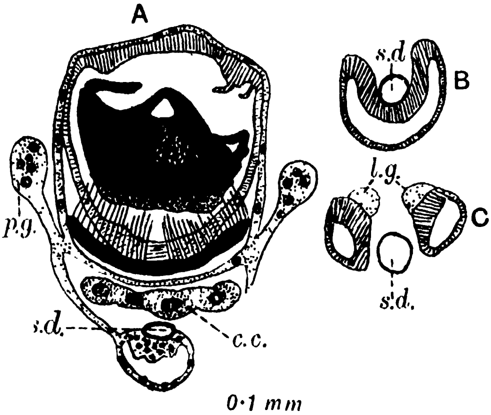

Fig. 13 A, B and C. The Labial Imaginal Disc as seen in the 30th, 32nd and 35th of a series of transverse sections 8μ in thickness.

The *Maxillary* discs are at first mere thickenings of the hypoderm in the base of the larval maxillae, but later they become true imaginal discs with peripodal cavities and membranes. The disc of the *Labrum* is possibly represented by a region on its dorsal surface where the hypoderm nuclei are crowded together. I have found no trace of *Mandibular* imaginal discs.

Text-fig. 14 A to F represents the 39th, 45th, 47th, 51st, 55th and 61st of a series of transverse sections through the imaginal sacs and rudiments of the antennae and eyes; A and B only show the sac of the right-hand side; C to F show both sacs; C is from a section through the extreme anterior end of the prothorax.

On each side of the head a deep dorso-lateral invagination of the hypoderm (*d.i.*) runs backwards from the base of the larval antennae (A). In places the two sides of the invagination are fused together so as to appear as a single hypoderm lamella. The base of the invagination swells out and constitutes the peripodal membrane (*p.m.*) of the Antennal Imaginal Sac through which runs the antennal rudiment (*a.*). The larval eye lies close to the lateral wall of the invagination but is entirely outside the cavity of the latter. The dorso-lateral invagination does not extend to the posterior border of the head though its ventral enlarged region is continued backwards into the thorax. Thus a transverse section through the posterior region of the head (B) shows the two imaginal antennal sacs with no apparent connection with the hypoderm of the head. In the anterior region of the prothorax (C) the two imaginal sacs lie dorsal to the gut, almost touching each other in the median line. Dorsally they are joined together by a transverse plate of hypoderm (*t.*) which is derived from the dorsal hypoderm at the junction of head and thorax and extends backwards into the prothoracic segment. In *Chironomus*, according to Miall and Hammond (17), there is, instead of a plate, a hypodermal fold enclosing a narrow space between its dorsal and ventral walls and extending back into the thorax, and the dorso-lateral invaginations of the cephalic

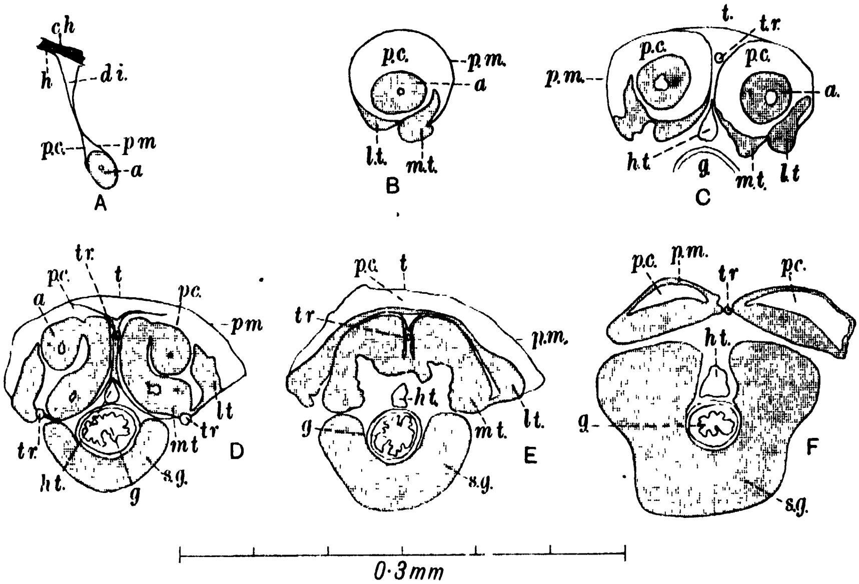

Fig. 14 A–F. The Imaginal Rudiments of the Eyes and Antennae as seen in the 39th, 45th, 47th, 51st, 55th and 61st of a series of transverse sections 8μ in thickness. *g.*, oesophagus; *tr.*, tracheal vessel; *h.t.*, dorsal vessel. For other lettering see text.

hypoderm extend right to the posterior border of the head and then backwards into the prothorax as invaginations of the ventral wall of the transverse fold. The transverse plate of *Forcipomyia* is probably a reduction of an original fold similar to that in *Chironomus.* In B and C may be seen two thickenings of the ventral wall of the imaginal sacs; these probably form certain regions of the imaginal head capsule in the neighbourhood of the eyes and antennal bases. The more median thickenings (*m.t.*) are directly continuous posteriorly with the bases of the imaginal antennae, the more lateral ones (*l.t.*) with the imaginal eye rudiments. Slightly anterior to the sub-oesophageal ganglion (*s.g.*) the median walls of the two sacs fuse together forming a thin vertical partition of imaginal cells.

At about the level of the anterior end of the sub-oesophageal ganglion the median part of the dorsal wall of the conjoined sacs loses its connection with the lateral regions of the walls (D). At this point the two sacs communicate with each other dorsally and are roofed over in the median region by the transverse plate of hypoderm mentioned above. Slightly more posteriorly the lateral thickenings of the ventral wall of each sac extend upwards as two plates of imaginal cells which unite in the median line immediately above the imaginal antennal bases which are thus excluded from the peripodal cavity (E). This is almost the posterior limit of the antennal rudiments, and it is at this point that the antennal nerves enter their bases. The peripodal cavity (*p.c.*) is now in the form of a transversely crescentic space whose thickened ventral wall is formed by the dorsal extensions of the lateral thickenings of the antennal imaginal sacs. This crescentic space is the Imaginal Sac of the Eye rudiments. Slightly anterior to the supra-oesophageal ganglion it becomes divided into right and left halves by the fusion of its dorsal and ventral walls in the median line. Each half consists of a peripodal cavity enclosed by a thickened ventral wall (which is the eye rudiment) and a thin and arched dorsal wall.

As may be seen from the figures the antennal rudiments extend as straight and thick-walled hollow cylinders through the whole length of the antennal sacs, their apices lying in the bases of the larval antennae. A nerve (not shown in the figures) runs through the axial cavity. In *Chironomus* the similarly disposed nerve is, according to Miall and Hammond (17), the larval antennary nerve, and is replaced in the imago by new nervous elements. I have not followed the later history of this nerve in *Forcipomyia.*

The Trunk Imaginal Discs consist of four in each thoracic segment and a median ventral one in the last abdominal segment. The points of invagination of the Leg discs are situated slightly posterior to the ventro-lateral setae, and just over each point of invagination is a group of three minute setae already mentioned as the vestigeal legs of the larva. The imaginal sac lies dorsal to its point of invagination, close against the hypoderm of the lateral body wall, and the leg rudiment arises as an ingrowth from the median wall of the sac.

In the mesothorax the imaginal sacs of the Wings are situated dorsal and slightly posterior to the mesothoracic leg sacs. They are dorso-ventrally elongated and lie close against, and their outer wall apparently fused with, the lateral hypoderm of the segment, their point of invagination being at their dorsal ends. Ventrally their peripodal cavity communicates with that of the leg sac. The wing rudiments arise as a ventrally directed infolding from the dorsal wall of the sac. Both leg and wing rudiments have a central cavity. That of the leg persists in the imago and contains the muscles and nerves, etc., of the leg; the cavity of the wing rudiment is only represented in the imago by the lumina of the wing veins. In the metathoracic segment the sacs of the halteres occupy a similar position and are of a like nature to the sacs of the wing discs.

In the prothorax there are two small discs in the positions occupied in the mesothorax by the dorsal ends of the wing sacs. These discs, which are the rudiments of the pupal respiratory trumpets, are at first mere lenticular thickenings of the hypoderm. Shortly before pupation they develop into hollow tubular *outpushings* of the hypoderm and come to lie between the larval hypoderm and the overlying chitin. They are never at any stage invaginated and have therefore no imaginal sac; they thus differ fundamentally from the other thoracic discs and cannot, I think, in spite of their position, be homologized with the imaginal discs of the wings and halteres.

In the last abdominal segment an imaginal sac is developed from the ventral hypoderm slightly anterior to the anus, and projects forwards below the rectum. It contains a longitudinal imaginal rudiment on each side and these are probably the rudiments of the External Genetalia.

I have not followed out the details of the *Muscular System* or the *Genital Organs*. The latter are easily recognizable in the earliest larval stages which I have examined, and have an uninterrupted development not undergoing the histolysis which affects in varying degrees the other larval tissues.

LIST OF LITERATURE.

(1) **BEAUREGARD**, H. (1890). *Les Insectes Vésicants.* (Paris.)

(2) **BECKER**, R. (1910). Zur Kenntnis der Mundteile und des Kopfes der Dipteren-Larven. *Zool. Jahrb. Abt. für Anat. u. Ont. der Thiere,* Bd. XXIX.

(3) **BERLESE**, A. (1909). *Gli Insetti,* I. (Milan.)

(4) **CARPENTER**, G. H. and **POLLARD**, F. J. S. (1918). The Presence of Lateral Spiracles in the Larva of Hypoderma. *Roy. Ir. Acad. Proc.* XXXIV, Sec. B, No. 4.

(5) **COMSTOCK**, J. H. (1920). *An Introduction to Entomology.* (Ithaca, N.Y.)

(6) **GEHUCHTEN**, A. **VAN** (1890). Recherches histologiques sur l'appareil digestif de *Ptychoptera contaminata. La Cellule,* T. VI.

(7) **HUFNAGEL, MME** A. (1918). Recherches histologiques sur la Métamorphose d'un Lépidoptère (*Hyponomeuta padella,* L.). *Arch. de Zool. Exp. et Gén.* T. 57.

(8) **IMMS**, A. D. (1907). On the Larval and Pupal stages of *Anopheles maculipennis,* Meigen. *The Journal of Hygiene,* VII, No. 2.

(9) —— (1908). *Ibid. Parasitology,* I, No. 2.

(10) **KEILIN**, D. (1915). Recherches sur les Larves de Diptères Cyclorhaphes. *Bull. Sc. de la Fr. et de la Bel.* 7ᵉ sér. T. XLIX, Fasc. 1–2.

(11) —— (1917). Recherches sur les Anthomyides à Larves Carnivores. *Parasitology,* IX, No. 3.

(12) **KOWALEVSKY**, A. (1886). Zum Verhalten des Rückengefässes und des guirlandenförmigen Zellenstrangs der Musciden während der Metamorphose. *Biol. Centralbl.* Bd. VI.

(13) —— (1889). Ein Beitrag zur Kenntnis der Excretionsorgane. *Biol. Centralbl.* Bd. IX.

(14) **LOWNE**, B. T. (1895). *The Anatomy, Physiology, Morphology and Development of the Blow Fly* (*Calliphora erychrocephala*). (London.)

(15) **MALLOCH**, J. R. (1915). The Chironomidae; or Midges of Illinois, with particular reference to the species occurring in the Illinois river. *Bull. of the Ill. State Lab. of Nat. Hist.* X, Article VI.

(16) —— (1917). A Preliminary Classification of Diptera, exclusive of Pupipara, based upon Larval and Pupal characters, with keys to Imagines in certain Families. Part I. *Ibid.* XII, Article III.

(17) **MIALL**, L. C. and **HAMMOND**, A. R. (1892). The development of the head of the Imago of *Chironomus. Trans. Linn. Soc. Lond.* V, part 8, 2nd ser. Zool.

(18) —— (1900). *The Structure and Life History of the Harlequin Fly* (*Chironomus*). (Oxford.)

(19) **PANTEL**, J. (1898). Le *Thrixion Halidayanum,* Rond. *La Cellule,* T. XV, 1ʳ fasc.

(20) **PÉREZ**, CH. (1902). Contribution à l'étude des Métamorphoses. *Bull. Sc. de la Fr. et de la Bel.* T. XXXVII.

(21) **PERRIS**, E. (1870). Histoire des Insectes du Pin maritime. Diptères. *Ann. Soc. Ent. Fr.* T. 4.

(22) **SEURAT**, L. G. (1899). Contributions à l'étude des Hyménoptères entomophages. *Ann. Sci. Nat. Zool.* 10.

(23) **VANEY**, C. (1902). Contributions à l'étude des Larves et des Métamorphoses des Diptères. *Ann. de l'univ. de Lyon,* Nouv. série I. Sciences, Médecine, Fasc. 9.

(24) **VAN REES**, J. (1889). Beiträge zur Kenntniss der inneren Metamorphose von *Musca vomitoria* (*Zool. Jahrb. Abt. für Anat.* III).

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FURTHER OBSERVATIONS ON THE RELATION OF THE HEIGHT GROWTH OF TREES TO METEOROLOGICAL CONDITIONS*

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(With 2 Graphs.)

I. Introduction. Several attempts have been made to estimate the influence of variable environmental factors in determining the rate of growth of trees by observing correlations between fluctuations in the rate of growth and corresponding variations in the intensity of the environmental factors. Fluctuation in the annual increments of trees can be observed either by measuring the widths of the annual rings of wood laid down in successive years or, in the case of conifers, by measuring the intervals between the annual whorls of branches; these fluctuations may be compared with weather records from neighbouring meteorological stations.

Various writers who have employed this method claim to have demonstrated correlations between annual increments and the following climatic factors:

(i) Rainfall and snowfall either during, or previous to, the growing period.
(ii) Air and soil temperatures during the previous winter.
(iii) Air and soil temperatures during the growing period.
(iv) Rainfall and temperature during July and August of the previous year.

(i) Henry (6) based his conclusions on observations in the neighbourhood of Nancy after the dry summer of 1893 and showed, for that year, that the annual rings of beech, oak, hornbeam and spruce were remarkably narrow, especially in trees growing on dry soils. Mer (10), also working at Nancy, measured annual rings and height increments of silver fir (*Abies pectinata*) for the years 1885 to 1894. The minimum annual rings were made in 1888 (a cold summer) and 1893 (a dry summer), and he concluded that whereas dry summers reduced both ring thickness and height increment, cold summers reduced ring thickness but not height increment. Bogue (2) measured the ring widths of forty-two trees of fifteen species for the years 1892 to 1904, and though no very definite relationship is visible in his figures, he reached the conclusion that abnormally high or low precipitations were evidenced by broad or narrow rings respectively in the *following* year. Stewart (14) found a general relation between the annual rings of a single oak and the rainfall for the years 1843 to 1893.

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* See Hiley and Cunliffe (8).

The most serious attempts to demonstrate a relationship between rainfall and annual ring thickness were made by Douglass (4, 5) on *Pinus ponderosa* in the dry climate of Arizona, and Huntingdon (9) on *Sequoia gigantea* in California. Douglass (5), p. 66, states that "the accuracy with which the pine trees near Prescott represent the rainfall recorded in that city for forty-three years is, without correction, about 70 per cent. By a provisional correction for conservation of moisture in the soil this accuracy rises to about 82 per cent." The degree of accuracy indicated in this statement is rather obscure.

(ii) A correlation between the widths of annual rings and winter temperature has been demonstrated by Schwarz (13) for *Pinus silvestris* in northern Prussia. Using the mean of thirty-one trees, the ring widths of which for the years 1879 to 1894 were measured by Schwarz, we have calculated a correlation coefficient of + 0·77 ± 0·07 with January-March mean air temperature and a coefficient of + 0·78 ± 0·07 with the soil temperature at 0·6 m. for the same period. From these figures it appears that in this instance winter temperature was more effective in causing fluctuations in ring widths than all other causes together. This occurred in a region in which the mean air temperature for the months concerned was 0·0° C. and the mean soil temperature 2·2° C. Schwarz ascribed the poor growth during seasons following cold winters to the lateness of the renewal of cambial activity.

(iii) Pearson (11) recorded a negative correlation between annual height increments of *Pinus ponderosa* and May-June temperatures and ascribed it to excessive transpiration during hot years, and Robbins (12) observed a negative correlation between ring widths of oak and May-June temperatures. A correlation coefficient of – 0·57 ± 0·11 has also been calculated between ring widths in *Pinus silvestris* as measured by Schwarz and May-June temperature, but since the latter showed a correlation coefficient of – 0·47 ± 0·13 with the January-March soil temperatures it is questionable whether much reliance can be placed on this relationship. The writers (8) found a coefficient of – 0·80 ± 0·07 between height increments of Corsican pine at Oxford and May-June soil temperatures at six inches depth, whereas the highest correlation with rainfall, namely that for the period April to June, was only + 0·63 ± 0·10.

(iv) Hesselmann (7) made observations on *Pinus silvestris* in Sweden during the years 1901 to 1903. The year 1901 was abnormally warm and dry, whereas 1902 was cold and wet. The shoot growth in 1902 was 50 to 100 per cent. higher than in 1901 and that in 1903 was abnormally low, facts which he ascribed in each case to the weather conditions during the previous year. Cieslar (3) obtained an apparently contradictory result, for after the dry hot summer of 1904 the height increment of spruce in Austria in 1905 was unusually low. These authors are of opinion that a correlation between annual height increment and the weather conditions in the previous summer can be explained on physiological grounds, since they consider that most of the growth in any year is performed at the expense of food reserves stored up in the previous year[1]. Cieslar considers that the opposite effect of a warm dry summer in the two countries might be due to the fact that Sweden and Austria belong to totally distinct climatic regions. In any case the observations cover periods too short to give reliable evidence and Cieslar's figures do not bear statistical analysis[2]. Cieslar failed to find any corresponding correlation between ring widths and the weather for the previous July and August.

The chief interest in the observations here summarised lies in the fact that with various species in different countries growth is found to depend on very different climatic factors, and in some cases on factors that would not be expected to exert any important influence. This suggests that a statistical study might provide useful information as to the climatic factors which limit the growth of a species in various parts of its range. Such information would be valuable to the silviculturist in deciding what species to plant in given localities, to the ecologist in the study of adaptation, and ultimately to the phytopathologist who will have to take into account the environmental limitations of a species when exploring the possibility of controlling its parasites. At present pathologists are handicapped by ignorance of the specific factors which control the growth of the hosts in different localities. The literature of the genus *Chermes* provides instances of the need for such studies.

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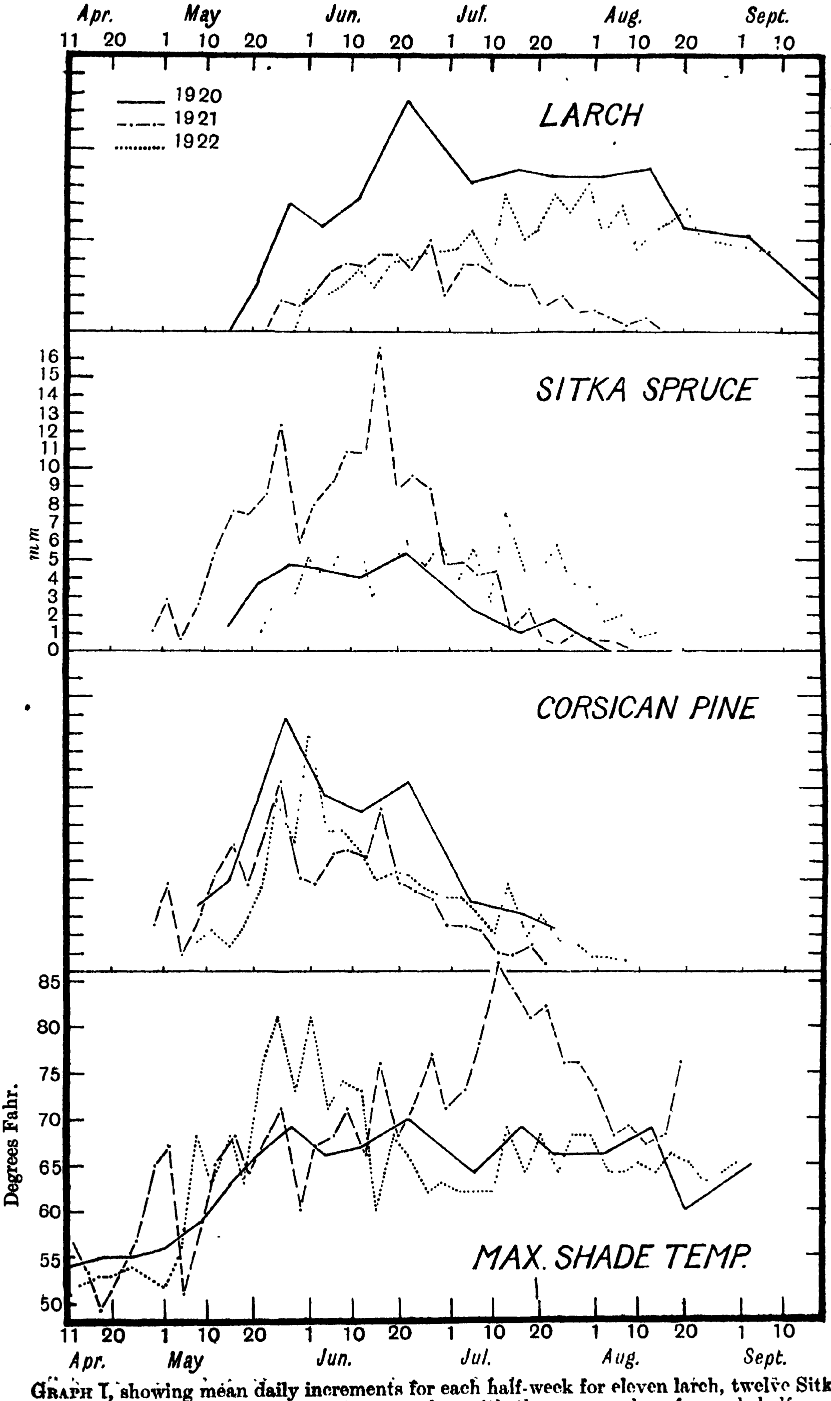
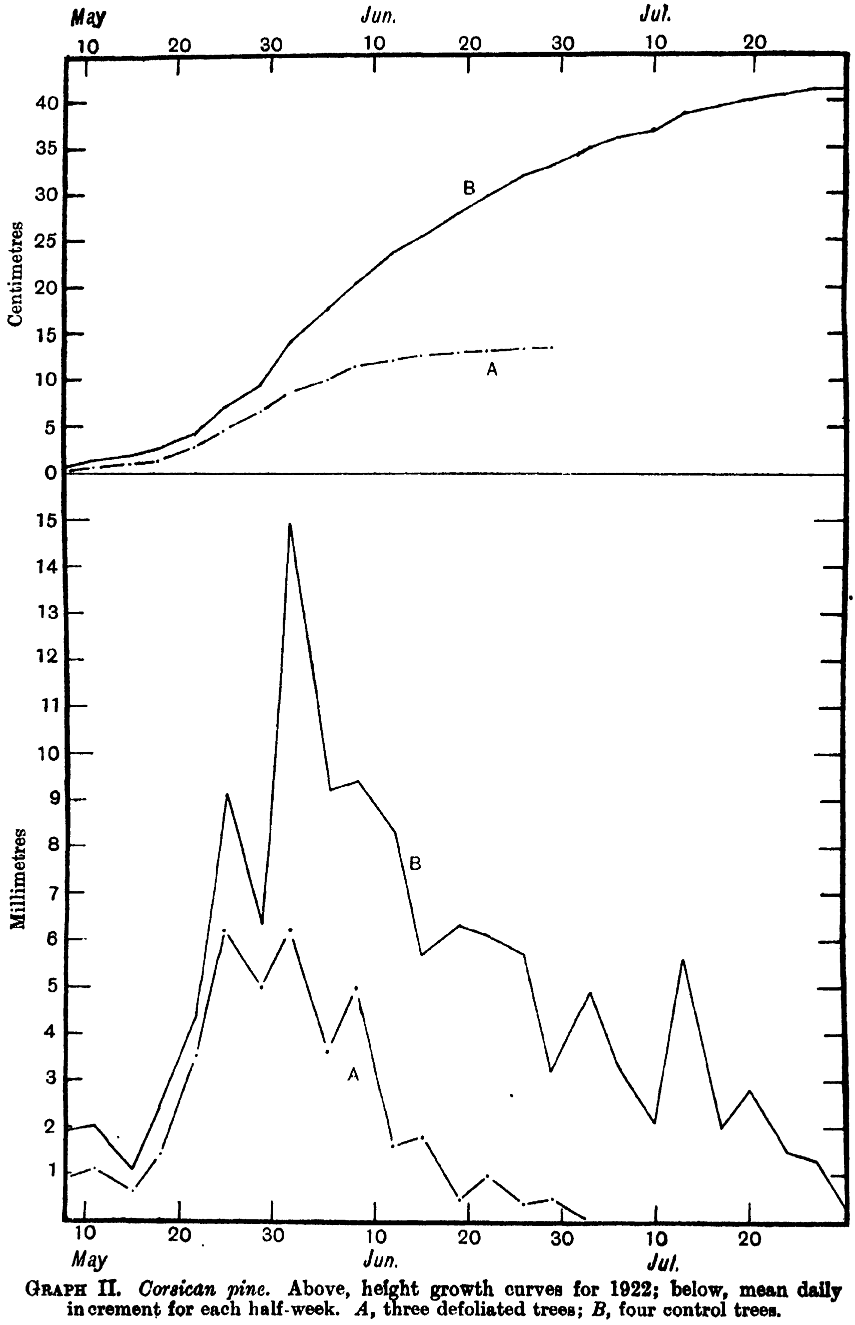
[1] See, however, p. 451 of this paper.

[2] Cieslar considers that the expression for climate which most directly affects growth is the "Vegetation quotient" $\left(\frac{\text{precipitation}}{\text{temperature}}\right)$. The correlation coefficient between annual height increment and the vegetation quotient for the previous July–August is only +0·14 for the four years covered by his observations.



GRAPH I, showing mean daily increments for each half-week for eleven larch, twelve Sitka spruce and eleven Corsican pine, together with the mean values for each half-week of the maximum shade temperature.



Graph II. *Corsican pine.* Above, height growth curves for 1922; below, mean daily increment for each half-week. *A*, three defoliated trees; *B*, four control trees.

The value of observations of annual increments would be greatly enhanced by a knowledge of the actual period in each year during which the increment was added[1], and the interpretation of such observations may only be possible when we know the direct effect of daily fluctuations in temperature, etc., on the daily increment. In the present paper the half-weekly increments of the terminal shoots of trees belonging to three species (European larch, Sitka spruce and Corsican pine) are recorded, and a comparison is afforded with similar increments for the two previous years already published (8). The results of measurement have been correlated with meteorological fluctuations.

Detailed descriptions of the sites, methods of measurement, etc., have been recorded in the previous paper (8). The same trees were under observation in 1922 as in 1921. Of the trees observed in 1920 only the Corsican pines were on the same site as those observed in the later years; the larch were growing under very similar conditions, but the Sitka spruce were on a drier and less favourable soil.

II. GROWTH PERIODS. The mean daily increments for each half-week of the growing season are shown in Graph I. Table I shows the dates of commencement of growth, maximum increment and completion of 80 per cent. of the year's growth. Table II shows the mean monthly temperatures from January to August for the three years, and Table III the monthly rainfalls.

Table I. *Period of Height Growth.*

		Larch	Sitka spruce	Corsican pine
Measurable growth commenced (*i.e.* more than 1 mm. per diem)	1920	May 21	May 15	Before May 4
	1921	May 26	April 28	April 28
	1922	June 1	May 22	May 8
80 % of growth completed by	1920	Aug. 17	June 29	June 29
	1921	July 25	July 11	July 4
	1922	Aug. 17	July 24	July 17
Max. rate of growth	1920	June 22	June 22	May 26
	1921	June 27	June 16	May 25
	1922	July 31	July 13	June 1

Table II. *Mean Monthly Temperatures in Degrees Fahr.*

	Jan.	Feb.	March	April	May	June	July	August
1920	42·1	42·8	46·0	47·2	54·7	58·7	57·5	56·4
1921	45·8	40·4	46·3	47·5	54·4	60·1	68·2	60·9
1922	39·5	40·3	40·4	42·6	57·4	58·4	57·4	56·9

Table III. *Monthly Rainfall in Inches.*

	Jan.	Feb.	March	April	May	June	July	August
1920	2·14	0·63	1·64	3·08	1·69	3·19	4·68	1·11
1921	2·46	0·56	0·93	0·91	1·50	0·34	0·28	1·65
1922	2·42	2·29	1·93	2·83	1·34	1·19	3·55	5·13

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[1] Antevs (1) gives a very useful bibliography of publications relating to growth in thickness.

Table IV. *Water Content of Soil*, 1922[1].

Plot	Nature of soil	Depth in inches	Water content in percentage of air dry weight of soil						
			May 4	May 18	June 8	June 22	July 6	July 20	Aug. 7
Sitka spruce	Clay	0–6	29	28	28	25	28	33	34
,, ,,	,,	12–18	16	26	21	19	24	24	Sat.
,, ,,	Peaty sand	0–6	130	154	49	?	145	90	160
,, ,,	,,	12–18	Sat.	Sat.	39	19	Sat.	34	Sat.
Larch	Gravel	0–6	34	28	18	5	26	25	39
Corsican pine	,,	0–6	17	16	5	5	9	12	—

III. THE RELATION OF DAILY GROWTH TO WEATHER.

(*a*) *Method of analysis of data.* In calculating correlation coefficients the following method was employed.

(i) The logarithms of the mean daily increments for the half-weekly periods were plotted. The mean of each successive set of five readings was taken and these mean points joined by straight lines.

(ii) The differences between the actual logarithms and the values read off from these straight lines were tabulated, the values outside the first and last means being omitted.

(iii) The climatic factors were treated in the same way except that direct values were used in place of logarithmic values.

(iv) Correlation coefficients between the increments for each species and each weather factor were calculated by the formula

$$r = \frac{\Sigma xy}{n\sigma_x \sigma_y}, \text{ error} = \pm \frac{0{\cdot}674\sqrt{1-r^2}}{\sqrt{n}}.$$

(*b*) *Results.* The correlation coefficients are shown in Table V in which the 1921 results are included for comparison. From this table it will be seen that in the case of both Sitka spruce and Corsican pine the results are remarkably similar in the two years. Fluctuations in the rate of growth were again determined principally by maximum or mean shade temperature. The effect of minimum temperature on the growth of Corsican pine appears from the table to be considerable, but no importance should be attached to this since it was found that the cross correlation between maximum and minimum shade temperatures *for the Corsican pine period* is + 0·48 ± 0·11.

The larch results for the two years 1921–2 are very different from each other. This may be ascribed to the hot weather in 1921 which, as shown in our previous paper, exerted an adverse influence on the growth of this

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[1] The samples were taken from the soil immediately adjacent to the sample trees.

Table V. *Correlation coefficients for* 1922 *compared with those for* 1921 (*the latter in italics*).

Period over which correlation was calculated	Sitka spruce June 1 to July 24	Corsican pine May 15 to July 24	Larch June 5 to Aug. 21	Max. shade temperature June 5 to Aug. 21
Max. shade temp.	+0·66 ± 0·09 *+0·73 ± 0·06*	+0·72 ± 0·06 *+0·88 ± 0·03*	+0·54 ± 0·10 —	— —
Mean shade temp.	+0·65 ± 0·09 *+0·68 ± 0·07*	+0·76 ± 0·06 *+0·79 ± 0·06*	+0·58 ± 0·09 *+0·13 ± 0·12*	+0·81 ± 0·05 *+0·91 ± 0·02*
Min. shade temp.	+0·37 ± 0·14 *+0·52 + 0·10*	+0·56 ± 0·10 *+0·47 ± 0·12*	+0·38 ± 0·12 —	−0·17 ± 0·14 *+0·59 ± 0·09*
Diff. between max. and min. shade temp.	+0·42 ± 0·14 *+0·37 ± 0·12*	+0·16 ± 0·14 *+0·60 ± 0·10*	+0·25 ± 0·13 —	+0·85 ± 0·04 *+0·60 ± 0·09*
Depression of wet bulb	+0·34 ± 0·15 *+0·20 ± 0·13*	+0·28 ± 0·13 *+0·23 ± 0·14*	+0·10 ± 0·14 —	+0·74 ± 0·06 —
Daily horizontal motion of wind	−0·39 ± 0·14 *−0·20 ± 0·13*	−0·35 ± 0·13 *−0·06 ± 0·14*	−0·13 ± 0·14 —	−0·14 ± 0·14 *−0·20 ± 0·13*
Hours of bright sunshine	+0·38 ± 0·14 *+0·37 ± 0·12*	+0·24 ± 0·14 *+0·44 ± 0·12*	+0·24 ± 0·13 —	+0·89 ± 0·03 *+0·43 ± 0·11*

species. The correlation coefficient with mean shade temperatures in 1922 was + 0·58 ± 0·09, which agrees very closely with the value of + 0·63 ± 0·11 obtained in 1920. A noticeable feature in 1922 was the comparatively high coefficient which was obtained with minimum shade temperature despite the fact that the latter showed no positive correlation with maximum shade temperature during the period concerned. It appears impracticable to trace any correlation between half-weekly rainfall and growth since the effects of rainfall are so protracted. The soil had become very dry on June 22 (see Table IV) but the subsequent heavy rain does not appear to have influenced growth.

The regression coefficients show that in Corsican pine the rate of growth in 1922 was doubled by an increase in maximum shade temperature of 15·4° F. or mean shade temperature of 13·3° F. as compared with 9·0° F. and 7·0° F. respectively in 1921. For Sitka spruce the corresponding values for 1922 were 13·6° F. and 11·0° F. and for 1921, 10·9° F. and 9·1° F. respectively. This year we have been able to obtain regression coefficients for larch which indicate that the rate of growth doubles with an increase of 20·5° F. in maximum shade temperature or 17·4° F. in mean shade temperature.

IV. Effect of the Artificial Defoliation of Corsican Pine. The food material from which the tree makes its annual increment is doubtless derived partly from the reserve stored through the winter and partly from current manufacture. As a preliminary attempt to distinguish between the relative value of these two sources three Corsican pines were artificially defoliated towards the end of April. Pines lend themselves to such treatment better than other trees since the leaves of the current year do not emerge from the sheathing scales of the dwarf shoots until the height increment of the shoot that bears them is complete.

In order to prevent undue wounding the old dwarf shoots were not torn off, but the needles were pinched off above the sheathing scales. Basal growth occurred and the needles had to be pinched off again on June 1, 12, 22, and July 3. On June 1 the needles had attained a length of 1 to 2 cms., and although they were then cut short they grew out to about 3 cms. by June 12. Hence it cannot be maintained that carbon assimilation was entirely prevented, though it was probably reduced to less than 10 per cent. of the normal.

The defoliated trees had shown normal growth during the four previous years, the mean annual increment having been 45·5 cms. for these trees and 45·2 cms. for four trees used as controls. However, the 1922 increment of the defoliated trees was 13·4 cms. whereas that of the controls was 41·2 cms. Thus defoliation reduced the increment to 33·3 per cent. As will be seen from Graph II, the two sets of trees started growth at the same time, and until May 29 the defoliated trees maintained a growth of 70·8 per cent. of the controls. After this date the growth of the defoliated trees slowed down very markedly and it was completed nearly a month before that of the controls.

V. Summary and Conclusions. (1) The years 1920 to 1922 show a large range of difference in temperature and rainfall; 1920 was a year of even temperature throughout the summer, 1921 was exceptionally dry throughout, and particularly hot in July, 1922 was exceptionally hot from May 21 to June 2 but subsequently resembled 1920. The trees that have been observed have been affected to very varying extents by these meteorological differences. Corsican pine behaved very similarly in each year, and its growing period may be regarded, in the neighbourhood of Oxford, as being approximately from May 1 to July 25. The tree appears to be capable of growing at a rate of 7·5 mm. a day at any time from May 20 to June 27 if weather conditions are favourable.

After the cold spring of 1922 Sitka spruce began growth nearly a month later than in 1921. A rapid growth of 7·5 mm. a day or more has been recorded at various dates between May 16 and July 13.

Larch grows, under favourable conditions, all through the summer, and rapid growth may be observed from the end of May to the end of August.

The results recorded in this paper show that caution is necessary in calculating correlation coefficients between annual increments and the weather during the growing periods owing to the variability of these periods. In general, May and June cover the active period for Corsican pine with fair accuracy, but the former month may, or may not, be significant for Sitka spruce, and in the case of larch, the weather during August and September may, or may not, be of importance in determining the annual increment.

(2) The measurements made in 1922 confirm the conclusion previously reached that temperature is the dominant factor in determining the daily fluctuations in growth. In 1921 this fact was obscured in the case of larch, apparently because growth was repressed by the very high temperature and low rainfall during the growing period. Corsican pine and Sitka spruce were less sensitive to temperature in 1922 than in 1921 as is shown by the fact that in 1922 it took a greater increase in temperature to double the rate of growth than was the case in 1921. Larch is less sensitive to temperature than either Corsican pine or Sitka spruce.

(3) The effect of artificial defoliation on Corsican pine was very marked. The height increment during the year of the defoliated trees was one-third of that of the controls. During the early part of the growing season the growth of the defoliated trees was closely comparable with that of the controls but the rate of increment fell rapidly. Defoliation must greatly reduce both transpiration and carbon assimilation. The fact that growth of defoliated trees was rapid during the early part of the season suggests that the reduction in transpiration was not a controlling factor, and the trees were not greatly affected by mechanical injury. The result is most easily explained on the supposition that the reserve food supplies which maintained growth during the early part of the season became exhausted by July 3. Even before this date the normal growth was dependent more on current manufacture than on reserve food. It thus appears that two-thirds of the annual increment is made at the expense, not of reserve material, but of food manufactured during the season. This result is in direct contradiction to Cieslar's contention cited on p. 444.

REFERENCES.

(1) **Antevs**, E. (1917). *Progressus Rei Botanicae*, v, 285–386.

(2) **Bogue**, E. E. (1905). *Monthly Weather Rev.* Washington, xxxiii, 250–1.

(3) **Cieslar**, A. (1907). *Centralbl. f. d. gesamte Forstwesen*, xxxiii, 233–246 and 289–311.

(4) **Douglass**, A. E. (1914). In Huntingdon, *Carnegie Publ.* Washington, No. 192, 101–121.

(5) —— (1919). *Carnegie Publ.* Washington, No. 289.

(6) **Henry**, M. (1894). *Comptes Rendus*, cxix, 1025.

(7) **Hesselmann**, H. (1904). *Bot. Centralbl.* Jena, xvii, 311–460.

(8) **Hiley**, W. E. and **Cunliffe**, N. (1922). *Oxford Forestry Memoirs*, No. 1.

(9) **Huntingdon**, E. (1914). *Carnegie Publ.* Washington, No. 192.

(10) **Mer**, É. (1895). *Jour. de Bot.* ix, 202 *et seq.*

(11) **Pearson**, G. A. (1918). *Jour. of Forestry*, xvi, 677–689.

(12) **Robbins**, W. J. (1921). *Univ. of Missouri, Agric. Expl. Sta. Res. Bull.* No. 44.

(13) **Schwarz**, F. (1899). Dickenwachstum und Holzqualität von *Pinus silvestris*, Berlin.

14) **Stewart**, M. N. (1913). *Monthly Weather Rev.* Washington, xli, 1287.

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REVIEWS

Practical Bee Anatomy. With Notes on the Embryology, Metamorphoses and Physiology of the Honey Bee. By ANNIE D. BETTS, B.SC. (LOND.). The Apis Club, Benson, Oxon, England. Pp. 76. 1923.

This little manual forms Volume I of "The Apis Club Library." The object of the series is to provide hand-books on the science and practice of Bee Culture in all its important phases. Apiculture, if it is to attain the status of a highly developed rural industry, needs the bee-keeper to acquire more exact knowledge of his subject and specialisation is also desirable in many cases. With these motives in view the publishers have decided to issue a number of volumes each complete in itself. We may say that the present volume forms a very thorough and exhaustive treatment of bee anatomy. It contains all the information that the most exact and enterprising bee-keeper is likely to demand on this subject. In fact it goes into far more detail than the majority of bee-keepers will attempt to assimilate. The professed biologist might also be well advised to turn to it, for he will find in its pages a thoroughly reliable exposition of bee-morphology with the most important literature quoted in the bibliography. The authoress is evidently well acquainted with the German language, and is able to lay before the reader the chief results of continental researches. The book contains useful notes on dissection, technique, etc., and there are a large number of figures grouped on twelve plates. These illustrations, unfortunately, are poor and sketchy and often difficult to interpret.

Manual of Entomology with special reference to Economic Entomology. By H. MAXWELL LEFROY, M.A., Professor of Entomology, Imperial College of Science. Illustrated by L. N. Stanilard. Pp. xvi + 541. 4 plates and 179 figures. London, Edward Arnold and Co., 1923. Price 35*s*.

In this book the author gives an account of the orders and the great majority of the families of insects, which cannot fail to be of service to students who, having acquired a ground-knowledge of structure and life-history, desire to pursue the systematic branch of the subject in detail. Professor Lefroy tells us in his preface that the volume "is based upon the lectures given as the second of three parts of a course occupying one year of a full training in economic entomology." The students who have received in the form of lectures the instruction comprised in this book, had doubtless, during the first part of their course, examined in the laboratory various insects both in the adult and larval stages, and had been thereby prepared for the professor's systematic teaching. It is to be regretted that the absence of the morphological and developmental details that are essential as an introduction to the study of entomology seriously reduces the value of the book to the elementary student, who, starting to work through it, will find himself confronted on the first page with a technical description of the Apterygota and a discussion of their affinities—a group of which his preliminary knowledge is likely to be of the slightest. Professor Lefroy admits that he has "dealt lightly with structure and morphology," but he, as an enthusiastic and successful teacher, must realise that some ground-work of general knowledge is an absolutely necessary preparation for the appreciation of the wealth of information here provided for ensuring the further progress of the earnest student. One result of this omission is that the reader is frequently confronted with dogmatic statements about relationships and lines of descent for which no justification is put forward, and from which many entomologists would dissent. Does not the author exaggerate the divergence between Apterygota and Pterygota in view of the close correspondences between a thysanuran and a mayfly larva, and can the Protura, with their heads devoid of feelers and their specialised piercing and suctorial jaws, be regarded as even "possibly the most primitive group" of the Aptera?

Professor Lefroy's plan of exposition is to give a very brief diagnosis, followed by a summarised account of each order and family, mentioning or describing particularly those families and in some cases species which are of economic importance. The information given will be of much value to the appreciative reader, because it comprises many facts not available in ordinary text-books. For example, the sections on the Acridiidae and the Termitidae contain an excellent summary of our knowledge for the student of bionomics and of economic zoology. Perhaps the book would have been more valuable from the latter standpoint had the author contented himself with a very brief reference to families of no economic importance and so found scope to describe more fully those insects which come into direct relation with human health or industry; the Scolytidae, for instance, cannot be effectively dealt with in the two pages allotted to them, and the Oestridae are dismissed in less than a page.

There is no formal bibliography, but references to authors' names with dates will enable a student, by use of the *Zoological Record*, to trace the papers quoted. In some cases, however, he will need to turn to the volume for the year following that mentioned in order to find the reference. The illustrations are clear and to a large extent original; some of them would have been improved in appearance had the drawings undergone more drastic reduction in the process-work. It may be presumed that the author intends to issue later the first and third parts of his course, and thereby afford those students who have not the privilege of his personal instruction an introduction to the whole subject and a detailed discussion of important economic species with directions for checking their ravages.

G. H. C.

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A STUDY OF THE FAUNA OF RIVERS POLLUTED BY LEAD MINING IN THE ABERYSTWYTH DISTRICT OF CARDIGANSHIRE

By KATHLEEN E. CARPENTER, M.Sc.
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(With 2 Maps.)

THE deleterious effect upon river fisheries of the discharge of effluents from lead mines into the rivers has long been recognised, and has even been made the subject of legislation (1); nevertheless, and notwithstanding the fact that definite recommendations for the control of such discharge have been issued by the Ministry of Agriculture and Fisheries, it is difficult to find evidence of any thorough scientific investigation of the subject, whose complexity is undeniable. Such an inquiry appeared to promise interesting results in the Aberystwyth District, whose main rivers have been affected for some hundreds of years by lead mining carried on beside their banks (2, 3), and the investigation was accordingly commenced in 1919, since which date it has been carried on continuously.

The area chosen for study includes a strip of coast bordering on Cardigan Bay, and traversed by several rapid streams, of which the chief are the Rheidol, the Ystwyth, and the Clarach. The two former have their sources in the Plynlimon Plateau, an elevated region of anticlinal folding along a north to south axis, whose highest point (Pen Plynlimon Fawr) rises to 2468 ft. The Clarach, a shorter stream, rises on the edge of the High Plateau west of the Plynlimon ridge, at an elevation of about 1200 ft. The lower courses of all three rivers are roughly east to west across the Coastal Plateau, which slopes to about 400 ft. near the coast, whence steep cliffs descend to the sea. The local rocks are grits, flags and shales of the Valentian and Bala series; metalliferous veins occur along fault lines usually running E.N.E. to W.S.W., and mainly situated in the upper parts of the basins of the principal rivers (3).

All the streams of the district are rapid in flow, often cutting deep gorges in the middle parts of their courses, and for the most part their beds are stony. The lower valleys are often carpeted with boulder clay, and there, in quieter reaches, the rivers are slightly turbid with the resulting mud, but as a general rule, the waters are clear and rapid, and the banks are strewn with gritty pebbles brought down by the rivers in time of flood.

The general physical character of these streams is such as to suggest that trout, salmon, etc., might be found in them in abundance, and indeed, the rivers Leri, Dyfi and Teifi, streams not far distant and remarkably similar to them in general character, have rich fisheries—yet the local rivers, Rheidol and Ystwyth, are absolutely destitute of fish-life. The Clarach, although a smaller stream, does contain a few trout, and sewin, or sea trout, may at times ascend all three rivers for a very short distance. Trout occur in relative abundance in the pools and streams of the High Plateau region, and in some of the tributaries of the larger rivers, and where this is the case the general fauna and flora are of a much richer type than in the Rheidol and Ystwyth. Streams classed as "barren" are recognisable on the most superficial inspection: their waters are clear down to the pebbles of the bed—there is only the slightest coating of moss or liverwort on any of the stones, and algal and phanerogamic vegetation are usually conspicuous by their absence, with the interesting exceptions of the algae *Batrachospermum* and *Saccheria* (Limaniaceae), which often flourish in such situations. Such scanty vegetation gives little opportunity for the accumulation of sediment, hence the extreme clearness of these streams save when they are rendered turbid by recent floods.

The barren character of such waters has always been locally ascribed, without much enquiry or definite evidence, to the influence of lead mining and crushing operations which were formerly very extensive in the district—a theory which appears to be borne out by comparison of the distribution of the lead workings with that of the barren waters. Other suggestions which might possibly be made in explanation of the existence of two distinct types of flora and fauna in the streams of a single area have been considered as follows:

A. Variations in the Substratum.

The local Silurian rocks are remarkably homogeneous in character throughout the area. Boulder clay, derived from the Plynlimon Plateau, is unevenly distributed over the area, but the mere fact of its occurrence in quantity in the valleys of Lower Clarach, L. Rheidol and L. Ystwyth alike is sufficient to discount the value of its presence as a determining factor in the issue involved.

B. Variations in the degree of acidity or alkalinity of the waters of the several streams.

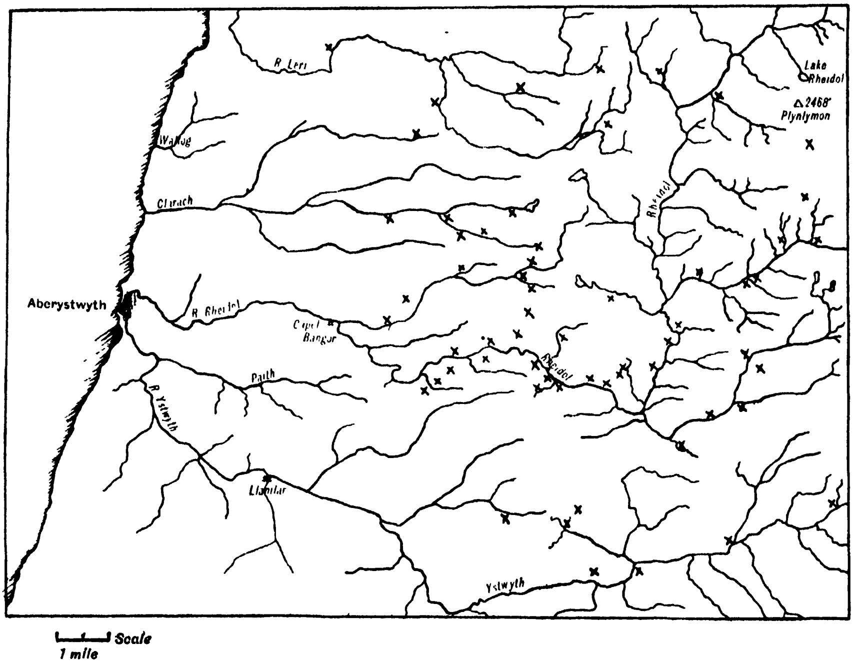
A series of water samples from representative streams within the area have been tested, and the degree of hydrogen-ion concentration determined by the colorimetric method established by Clark and Lubs (4). The general result of these investigations is to shew that the pH values of the waters of the various streams vary between 6·1 and 6·9, indicating slight acidity in every case, with no special line of demarcation between rich and barren streams. Examples may be given:

L. Rheidol (*barren*)	pH 6·4	L. Clarach (*rich*)	pH 6·4
L. Ystwyth ,,	,, 6·5	Bow Street ,,	,, 6·5
Nant Silo ,,	,, 6·6	Wallog ,,	,, 6·8

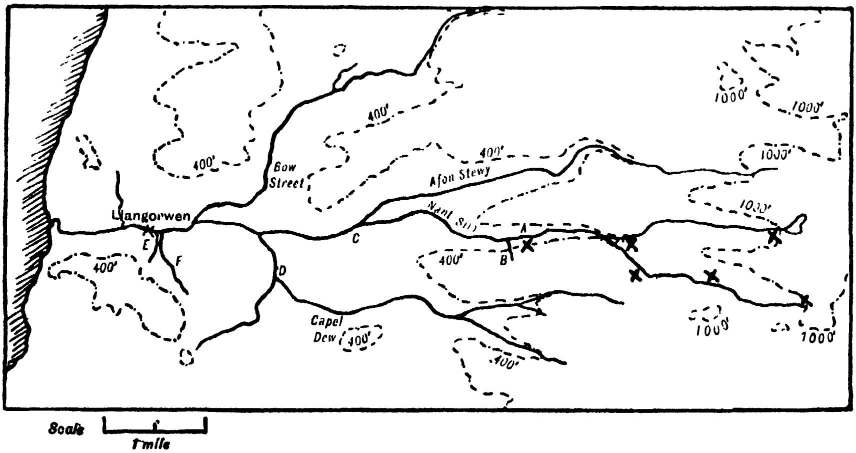
(The constant slight acidity, correlated with the scarcity of lime salts, is no doubt connected with a general poverty which characterises the freshwater fauna of the whole district, and which affects particularly the group Mollusca; but this general poverty is in no wise comparable with the special and remarkable poverty of the streams classed as "barren.")

There remains the argument that pollution consequent upon lead mining operations has seriously affected the fauna of certain waters. A glance at the map (p. 4) shewing distribution of Cardiganshire lead mines reveals the crowding of such workings around the head waters and many of the tributaries of Rheidol and Ystwyth: even this map is not quite complete, as there are many small mines unmarked upon the Ordnance Sheets, which could not in all cases be represented. Certainly all the lead mines have ceased working, many of them a considerable number of years ago, but possibilities of pollution from this source still remain. All the mine workings are situated near the banks of some stream, into which water from the mine effluents runs directly; in addition, there are open heaps of mine refuse containing material in all states of division, from rough blocks to fine sediments, much of it rich in lead ore (which occurs in the form of galena—PbS), and often covering a very considerable area of ground in the river valley. Surface drainage and wind action continually bring material from these heaps into the rivers, and that this material is actually still rich in lead is amply proved by a few chemical analyses of samples taken from the heaps. Two examples may be given: at Old Goginan Mine, a large working closed since 1886, material collected on November 1st, 1922, from the side of

Distribution of Lead Mines in N. Cardiganshire.



Valley of the Clarach.



Contour lines —•—•— at 400′ and 1000′. Localities: A, B, C, D, E, F.
Lead mines: ×.

a mine tip facing the stream, was found on analysis to contain a percentage weight of lead amounting to 1·64 per cent. of the total weight of the dry sample. At Llywernog (closed 1883), a similar sample had percentage weight of lead 1·15. While samples taken from different parts even of the same heap may vary considerably in quality, yet there is obviously a considerable amount of lead ore present in the débris of the old workings—quite sufficient to cause serious pollution of the rivers, even if we neglect the fact that it was the ancient practice to tip the mine refuse directly into the neighbouring streams.

The topographical relationship between distribution of mine workings and distribution of barren waters has already been mentioned: an intensive study of the streams of the Clarach Basin, as reproducing in epitome many of the characteristic features of the wider area, makes this relationship particularly clear(5) (see map, p. 4).

Of the two head streams of the Clarach, Afon Stewy and Nant Silo, the latter, with its tributary from Cwm-erfin, flows through a district where lead mining has been carried on since very early times, the former, like the later affluents, Capel Dewi and Bow Street Brooks, is free from such contamination. The main stream of the Lower Clarach flows due east to west through a fine glaciated valley. A series of collections made in the area between the years 1919 and 1922 forms the basis of the following list, from which all mention of Protozoa, Nematoda and Rotifera has been omitted:

LOCALITY A. Nant Silo, near mine-effluent.

Animal species 3 (larvae).

Diptera	2	*Simulium latipes* *Tanypus nebulosus*
Ephemerida	1	*Chloeon simile*

LOCALITY C. Lower down the same stream, after the confluence with unpolluted Afon Stewy.

Animal species 7.

Diptera	2	Plecoptera	2
Ephemerida	2	Trichoptera	1

LOCALITY D. Unpolluted Capel-Dewi Brook.

Animal species 23.

Diptera	4	Arachnida	2
Ephemerida	2	Crustacea	1
Plecoptera	3	Oligochaeta	1
Trichoptera	5	Mollusca	1
Coleoptera	2	Chordata	2 (*Salmo fario*, *Gastrosteus aculeatus*)

LOCALITY E. Main stream at Llangorwen; far below any lead mines, and after union with several unpolluted tributaries.

Animal species 47.

Diptera	4	Crustacea	7
Ephemerida	2	Oligochaeta	1
Plecoptera	3	Hirudinea	1
Trichoptera	9	Mollusca	3
Coleoptera	8	Platyhelmia	2
Hemiptera	3	Chordata	2
Arachnida	2		

LOCALITY G. Bow Street Brook (unpolluted). Fauna list intermediate between those of D and E. (Larger brook than Capel Dewi.)

Even the small brooks labelled B and F have a richer fauna than the much larger stream at A and C; at B, a mere impermanent trickle of running water, a few minutes' search sufficed to find 8 species, while at F, 11 are represented.

Poverty of fauna in the Clarach Basin is obviously related to some factor other than the mere physical geography, and proximity of lead mines appears to be very clearly indicated as this factor, here as elsewhere in the Aberystwyth district.

A comparison between fauna lists established for various localities between the years 1919 and 1922 is interesting. The localities quoted are as follows:

A. Lower Rheidol. A fair sized river, heavily polluted by numerous lead workings.

B. Lower Ystwyth. Similar to A.

C. Lower Clarach. A smaller river, some of whose upper waters are polluted in this way, but which has several pure affluents whose total volume exceeds that of the polluted waters. Such lead workings as occur are of less recent date than those affecting Rheidol and Ystwyth.

D. Wallog Brook. A much smaller stream, totally unpolluted, very stony.

E. Llanychaiarn Brook. A tributary of the Ystwyth, totally unpolluted; has alternations of mud and stony bottom.

(The actual names of species identified are omitted from these lists, for the sake of brevity.)

In the most thoroughly polluted waters, right under the lead mines, life is not absolutely extinct: larvae of *Tanypus nebulosus*, of *Simulium latipes* and of the Ephemerid *Chloeon simile* may usually be collected here, these species appearing particularly resistant to the dangers of

	A. L. Rheidol	B. L. Ystwyth	C. L. Clarach	D. Wallog	E. Llanychaiarn
Turbellaria	—	—	×	× ×	× ×
Mollusca	—	—	× × ×	× ×	× × ×
Oligochaeta	—	—	×	×	× ×
Hirudinea	—	—	×	×	×
Crustacea-Entomostraca	× ×	× × ×	× × × × × ×	× × × ×	× × × ×
Crustacea-Malacostraca	—	—	×	×	×
Arachnida	×	×	× ×	×	×
Hemiptera	×	× ×	× × × ×	—	—
Trichoptera	—	—	× × × × × × × × ×	× ×	× × × × × × ×
Plecoptera	×	× ×	× × ×	× ×	× × ×
Ephemerida	×	×	× ×	× ×	×
Neuroptera	—	×	—	—	—
Odonata	—	×	—	—	—
Coleoptera	× ×	× ×	× × × × × × × ×	×	× × ×
Diptera	×	×	× × × ×	× × ×	× × × × × ×
Chordata	—	—	× ×	—	×
Total	9	14	47	22	35

Number of animal species indicated by ×'s.

their circumstances, and in the summer the Hemiptera *Velia currens* and *Sigara minutissima* appear also.

The complete absence from seriously polluted streams of Platyhelmia, Mollusca, Chordata, Trichoptera, Crustacea-Malacostraca, Oligochaeta and Hirudinea is always remarkable, the fauna of such streams being limited to a few insect larvae and a few Crustacea-Entomostraca, which occur usually in very small numbers.

The economic, as well as the scientific, interest of the study of the biology of lead-polluted streams is considerable, in view of the financial losses caused by such pollution: even to the casual observer, the desolation of these waters suggests the desirability of protecting the streams in districts where fresh mines are being opened or where the consequences of mining activity are not yet too serious for correction. Any such protective measure in the interest of river fisheries must, if it is to be effective, be based upon thorough investigation of the manner in which lead pollution affects the whole population of the stream—not merely the fish themselves, not merely the fishes and their food organisms, but all the organisms which coexist with them, and all of which must be regarded as affecting the general equilibrium of life in the streams.

The first steps in such an investigation in the Aberystwyth district, which led to the establishment of comparative tables of river fauna, some of which are quoted above, established the conclusion that lead pollution serves to impoverish the whole fauna of the streams, affecting all groups in general, and tending to eliminate altogether the Fishes, Platyhelmia, Mollusca, Annelida and Trichoptera. The actual nature of the selective factor and its mode of operation could only be sought by inquiry into the chemical and physical conditions set up in the streams themselves.

Direct evidence of immediate effects of the discharge of mine effluents into hitherto unpolluted streams could not be collected in this district, as no new lead mines have been opened recently, but the conditions consequent upon such discharge continue to prevail in some cases for a number of years and may be summarised as follows. First, a large amount of solid mine débris containing galena particles in various states of division, from quite large particles to very fine gritty dust, finds its way into the rivers in the fashion already described. Second, the river water in its natural state is capable of exerting considerable chemical action upon the lead ore, with the result that lead compounds (probably the oxide and hydroxide) are found in actual molecular solution and also in the colloid condition in the waters thus affected. In times of flood, the waters of lead-polluted streams near Aberystwyth have been seen to have an opalescent appearance from the presence of material in the colloidal state, and plants growing on the banks appear slightly coated with slime after subsidence of the floods.

The presence of lead salts in solution (molecular and colloidal) in the polluted waters has been demonstrated by chemical tests which have been made incidentally to the biological investigation: of the actual chemistry of these compounds little can be said, as chemical specialists do not appear to have paid much attention to these phenomena, but it seems probable that the action of river waters on galena particles has many analogies with that of natural waters on lead foil, which has been so thoroughly investigated by Dr J. C. Thresh, of the Counties Health Laboratory (6). Dr Thresh's recent work has exploded the old theory that the plumbo-solvent power of water depended upon its acidity, and has established the importance of the presence of silicic acid and silicates as retarding agents in the process of lead solution. The character of such waters in the Aberystwyth district as have been proved to contain dissolved lead salts is such as to support the probability of the analogy suggested, these waters being unusually free from the usual dissolved salts, including silicates.

There would thus appear to be two possible sources of danger to the population of lead-polluted streams—danger from the presence of gritty particles of lead ore, such as might cause mechanical injury to delicate tissues, and danger from the presence of lead salts in diffusible form (whether in actual molecular solution or in "colloidal solution"), such as might exert either a chemical action from without upon epithelial tissues and secretions, or a toxic action from within after absorption. In the first case, probably mechanical injury alone need be considered, since it is well known that large amounts of lead in solid form may pass through the body after being swallowed, with practically no absorption, even under the action of the digestive fluids, and without fatal effects. This has been clearly shewn by Dr T. W. Hogg in the case of earthworms (7).

Two lines of investigation seem indicated. The possibility of mechanical injury from the presence of gritty particles is the only one to which serious attention has hitherto been paid by those interested in the preservation of river fisheries: the general theory has been that fishes are directly affected by the stirring up of rough galena grit from the river bottom, particles of which adhere to the gill filaments and there set up inflammation which ultimately causes death. On this theory, and on this alone, are based the recommendations framed by the Ministry of Agriculture and Fisheries for issue to owners of lead mines, in which recommendations there is insistence upon a method of sedimentation by passing the water of the mine effluent through tanks. In these tanks it is allowed to stand until sedimentation is considered to be complete, then run off from a high level. This is simply the equivalent of a rough filter method.

In support of the general theory referred to, I am permitted to quote evidence collected by Prof. Fleure at the time of the opening of the Hafan mine affecting the Leri in 1913; his statement is as follows:

"I found that these fish had rough grit and galena fragments between the gill filaments, and that these fragments had set up inflammation. A post mortem did not reveal anything wrong internally."

Further observation along these lines is desirable, but difficult to carry out, as it is by no means every day that a new mine is opened, affecting a hitherto unpolluted stream.

Indirect evidence has been sought in another way: by the quantitative chemical analysis of samples of finely divided grit, capable of being carried in suspension by swiftly moving water. These samples were obtained from the beds of local streams by dragging along in an upstream direction a small bag of silken tow-netting in a rigid frame, held in such a position that one side of the square frame just touched the surface of the bed.

The results of analysis may be tabulated as follows:

Locality	Description	% weight of lead in dry wt. of whole sample
A. L. Rheidol (Pen y Bont)	Fauna and flora extremely poor. Streams directly affected by extensive mining operations above these points	0·085
B. L. Ystwyth (Rhyd y Felin)		0·01
C. L. Clarach (Llangorwen)	Rich flora and fauna. There are lead workings near some of the higher tributary streams	0·12
D. Nant Silo	Fauna extremely poor. Sample taken just below an old lead working. This is a tributary of the Clarach	0·035
E. Gogerddan	Lower down the same tributary, after confluence with an unpolluted stream. Fauna intermediate	0·015
F. Wallog	Large brook—no lead mines affecting it. Fauna relatively good	0·01
G. Llanychaiarn	As above	0·012
H. Plas Hendre	A mere trickle of water, almost a roadside ditch	0·005

It should be noted that these figures are not absolute in themselves: a sample taken at a few yards' distance or at a day's interval might have yielded quite a different figure, nevertheless the records serve to establish two conclusions:

(1) Lead ore is present in gritty particles in the beds of streams which are absolutely unaffected by mining operations (it is in such cases certainly derived directly from the substratum, whether from the bed rock or from drift deposits).

(2) The poverty of fauna in the streams classed as "lead polluted" cannot be directly related to the presence of grit of this kind.

As regards the fishes themselves, we have the direct evidence quoted above that adhering gritty particles have been known to cause inflammation of the gills; but the question remains whether such a condition is referable to the nature of the particles, as galena fragments (in which case some kind of chemical action seems presupposed, the lead passing into diffusible form), or whether the injury is merely mechanical, and thus one to which fishes are exposed in any stream which bears along gritty fragments of whatever chemical nature—for example, in the neighbourhood of quartzite quarries. Of injury to fishes in such cases as the latter I have no evidence. At all events, the fact that trout, sticklebacks and elvers are found living in streams of the Aberystwyth district where a proportion of the sediment can be proved to consist of gritty fragments of lead ore (notably in the Clarach at Llangorwen, where both *Salmo fario* and *Gastrosteus* occur), proves indisputably that fishes are capable of existing under such conditions.

As regards the invertebrate population, the same argument holds good, nor is it reasonable to suppose that the larvae of Trichoptera such as *Limnophilus*, in their protective cases through which the respiratory current is drawn by a fine perforation in the closing membrane, nor the more minute and delicate species of aquatic animals, which escape many dangers by reason of their smallness, can be very seriously in danger of mechanical injury from rough grit.

So far as direct evidence goes, nothing more can be asserted with regard to the significance of the presence of lead ore in gritty particles than that the observed facts in the Aberystwyth district clearly shew that such a condition is by no means incompatible with the existence of a relatively rich fauna, including fishes and several of their more important food-organisms.

A second possibility remains: that the presence of lead salts in diffusible form (molecular or colloidal solution) may act as a selective factor upon the natural fauna of the streams. The toxic action of lead in such diffusible form when absorbed into the system has been studied in the human subject by various workers, and in the cases of chronic lead poisoning or "plumbism" which ensue upon such absorption the destructive action is extremely slow and the amounts absorbed into the system are relatively small (8). The suggestion of a similar action upon river fauna is an attractive one.

Few workers have investigated the action of dissolved lead salts upon animals immersed in water: the researches of Drs Penny and Adams in 1863 established the fact that lead acetate in a proportion of 1 to 4000 (*i.e.* 250 milligrammes per litre) is directly fatal to minnows and goldfishes (9). More recently, Mr J. Rushton (10), working with solutions of lead nitrate, records a strength of 1 in 100 as fatal to trout within 15 minutes, a smaller proportion of 1 in 100,000 having similar effects in 2 hours and 15 minutes.

Several facts should be noted in connection with these experiments:

(1) In each case the solution used is artificial in character: lead nitrate and lead acetate are convenient for laboratory use, being readily soluble, but it is not in such form that lead salts occur naturally in waters of mining areas.

(2) The proportions used, even in the last-quoted instance, are relatively enormous, and such as are not likely to occur in any stream under ordinary conditions. The reaction thus becomes rapid, and the experiments afford no parallel with lead poisoning of the cumulative type, known in the human subject.

(3) There has been no use of "colloidal lead" such as occurs in natural waters below the mines.

(4) Fish have been the sole subjects of experiment; their invertebrate food organisms have not been exposed to similar tests.

These experiments can therefore throw no light on the topic under discussion, except in so far as they establish definitely the contention that the presence of lead salts in solution may have a fatal effect upon Fishes.

In order to discover whether any correlation might be supposed to exist in the Aberystwyth area between impoverishment of fauna and presence of lead salts in diffusible form, water samples were collected from streams of very different types and a series of estimates of amount of lead present "in solution" (including molecular and colloidal solution, where the latter will pass through a filter paper), were made by the method advocated by Clowes and Colman (11).

The early series of determinations—1921 to spring of 1922 were as follows:

Date	Locality	Lead (mgr. per litre)	Type of fauna
Feb. 16–20, 1921	1. Lower Rheidol	0·2	A
	2. Lower Ystwyth	0·5	A
	3. Upper Clarach (at Nant Silo lead mine)	0·2	A
March 1–15, 1921	4. Llanychaiarn	—	B
	5. L. Ystwyth	0·4	A
	6. L. Rheidol	0·5	A
	7. Elysian Grove	—	B
	8. Lower Clarach	—	B
March 20, 1922	9. Wallog	—	B
„ 23, „	10. L. Rheidol	0·2	A
„ 27 „	11. L. Ystwyth	0·4	A
„ 29, „	12. L. Clarach	—	B
„ 29, „	13. Nant Silo	0·5	A

These figures seemed to indicate a definite relation between presence of lead in diffusible form and poverty of fauna (in waters of type A—hereafter referred to as the "polluted" type), contrasted with comparative wealth of fauna in streams of type B ("non-polluted"). So far, all the tests made shewed lead in readily diffusible form to be invariably present in streams of type A, although a comparison of figures (1), (6) and (10), or of (2) and (11) gave evidence of some variation in amount present. This latter fact did not affect the general relation stated above, and it seemed probable that the actual determining factor in the impoverishment of lead-polluted streams was presence of lead salts in diffusible form rather than presence of galena grit, although the latter might perhaps be directly harmful in some cases.

In the late spring of 1922, a long period of drought followed upon a winter of, on the whole, sub-normal rainfall (see rainfall tables). Probably also the water shortage consequent on the long summer drought of 1921 had not been made up: however this may be, in May and early June of the year 1922 the rivers of this district were very much lower than had been the case for many years, even in the preceding summer. By the end of May, many of the smaller brooks were altogether dried up, and the larger rivers in spite of their numerous tributary streams rising in the High Plateau, were greatly diminished in volume. In many parts of Lower Rheidol and Ystwyth the stream was seen to occupy only about half of its usual bed, exposing broad, flat surfaces of pebble deposits, and it was almost possible to walk dryshod from boulder to boulder across the Rheidol, our largest river, only a mile and a half above the sea. At this spot (near Pen y Bont), where usually a clear stream rushes over smooth boulders and pebbles, only a few of which are overgrown with Bryophytes or with the Alga *Saccheria,* masses of aquatic Phanerogams (*Ranunculus aquatilis* and *Callitriche verna*), made their appearance in the main stream and rapidly increased in size, while the usual Algal and Bryophyte vegetation was considerably augmented.

Observation of these changes suggested the advisability of making a careful re-examination of the fauna, which also was found to have undergone very considerable alteration, as shewn by the comparative table on p. 14.

(N.B. The fauna is in all cases that of the main stream, and not that of either temporary or permanent "cut-offs," which may be understood to be influenced by conditions different from those affecting the river itself.)

The total number of species had thus more than doubled itself; but a mere list of species can give no idea of the astonishing character of the change. A locality where on previous visits a few specimens only had with difficulty been collected now exhibited a positive wealth of animal life: not only did the new species establish themselves in considerable numbers, but species previously recorded (e.g. *Chloeon simile* and *Tanypus nebulosus*) were now represented by multitudes of individuals. The most remarkable feature of the change was the incursion of eight species of Trichopteran larvae into a region where they were hitherto unknown (at any rate since 1919, up to which date no records existed). Almost every pebble picked at random out of the river bed bore the pebble cases of *Rhyacophila* or of *Agapetus*; the minute horn-

Fauna of the Lower Rheidol.

	Species recorded previous to spring, 1922		Species collected in and after May, 1922	
Platyhelmia	—	0	Polycelis nigra	1
Crustacea	Cyclops serrulatus		Cyclops serrulatus	
	C. prasinus		C. prasinus	
	—		Canthocamptus minutus	
	Eurycercus lamellatus ...	3	Eurycercus lamellatus ...	4
Arachnida	Sperchon glandulosus ...	1	Sperchon glandulosus ...	1
Hemiptera	Notonecta glauca		Notonecta glauca	
	Sigara minutissima ...	2	Sigara minutissima ...	2
Coleoptera	Dyticus marginalis		Dyticus marginalis	
	Hydroporus rivalis		Hydroporus rivalis	
	—		Hydraena riparia	
	—	2	Gyrinus natator ...	4
Plecoptera	Chloroperla grammatica		Chloroperla grammatica	
	Leuctra nigra ...	2	Leuctra nigra ...	2
Ephemerida	Chloeon simile		Chloeon simile	
	—	1	Ecdyurus venosus ...	2
Neuroptera	Sialis flavilatera ...	1	Sialis flavilatera ...	1
Odonata	Platycnemis pennipes ...	1	Platycnemis pennipes ...	1
Diptera	Tanypus nebulosus		Tanypus nebulosus ...	
	—		Simulium reptans	
	—	1	S. latipes	3
Trichoptera	—		Rhyacophila obliterata	
	—		Agapetus fuscipes	
	—		Crunoecia irrorata	
	—		Hydropsyche angustipennis	
	—		Plectrocnemia conspersa	
	—		Anabolia nervosa	
	—		Limnophilus decipiens	
	—	0	Mesophylax impunctatus	8
	Total number of species	14		29

shaped tubes of *Crunoecia* clustered in vast numbers around the tufts of Water-Starwort; and in some places the bed of the river, usually clear and light owing to the pebbles which strew it, shewed dark brown shoals of *Anabolia* and *Mesophylax* through the water. The change was so striking that any description of it after the event has an effect of exaggeration: still, there remains the no less striking corroborative fact of the sudden establishment of aquatic flowering plants, noticed also by members of the Botanical Department of the University College and commented upon even by the unscientific observer.

The new record of *Polycelis nigra* is also interesting, this being the first Planarian found in this locality. A very marked feature of the change in general was the introduction of species of which the majority evince a direct dependence upon the aquatic vegetation, *e.g.* Caddis larvae such as *Anabolia*, which make their tubes of vegetable matter, *Polycelis*, which crawls over the leaves of water plants, *Canthocamptus* and *Hydraena*, which shelter among the tangle of fronds.

In the early days of the invasion there seemed to be a slight shortage of vegetable matter in proportion to the new animal population: this was illustrated by the behaviour of certain of the Caddis larvae which make their cases of vegetable fragments. Many *Anabolia* were found at this time (about June) with the posterior half of the case shewing the normal structure, the anterior portion a patchwork of small, flat pebbles, or even of the cases of smaller members of the species, whose occupants were still living.

The new conditions in the Rheidol were traced 5½ miles up stream to Capel Bangor.

In the Ystwyth, between Llanilar and Rhyd y Felin, an analogous series of changes took place. Among the flora, the incursion of Chlorophyceous Algae, forming tufted growths upon the pebbles, was particularly marked; the new fauna list presented 25 species as against the 9 of previous records, and here again one Planarian (in this case, *Dendrocoelum lactum*) made its appearance, while the new Trichopteran species numbered 7, the list differing slightly from that established for the Rheidol.

Fauna of the Lower Ystwyth.

	Species collected previous to May, 1922		Species collected in and after May, 1922	
Platyhelmia	—	0	Dendrocoelum lacteum	1
Crustacea	Cyclops serrulatus		Cyclops serrulatus	
	Eurycercus lamellatus	2	Eurycercus lamellatus	2
Arachnida	Sperchon glandulosus	1	Sperchon glandulosus	1
Hemiptera	Notonecta glauca		Notonecta glauca	
	—		Gerris paludum	
	—	1	Sigara minutissima	3
Coleoptera	Dyticus marginalis		Dyticus marginalis	
	Hydroporus rivalis		Hydroporus rivalis	
	—		Hydraena riparia	
	—		Elmis volckmari	
	—	2	Limnius tuberculatus	5
Plecoptera	Leuctra nigra		Leuctra nigra	
	—		Chloroperla grammatica	
	—	1	Isogenus nubecula	3
Ephemerida	Chloeon simile		Chloeon simile	
	—	1	Ecdyurus venosus	2
Diptera	Tanypus nebulosus		Tanypus nebulosus	
	—	1	Simulium latipes	2
Trichoptera	—		Rhyacophila obliterata	
	—		Agapetus fuscipes	
	—		Silo nigricornis	
	—		Brachycentrus subnubilus	
	—		Plectrocnemia conspersa	
	—		Hydropsyche angustipennis	
	—	0	Mesophylax impunctatus	7
	Total number of species	9		26

In both rivers, the new condition had all the characteristics of an invasion by a population coming from the smaller tributary streams, which support a flora and fauna usually unable to exist in the main river. This was particularly well illustrated round about the confluence with the Ystwyth of the Llanychaiarn brook, which latter, though of no great size, has a relatively rich fauna. At the season mentioned, the brook was almost dried up, but stones picked from the bed of the river near the confluence (above as well as below, though in a lesser degree) were thickly set with cases of *Agapetus* or of *Hydropsyche*, both of which are at all seasons common in the brook. In all probability there was direct immigration of individual larvae from their original homes: the date of the incursion makes this obvious, as all the larvae were found in May, whereas in no case except that of *Brachycentrus subnubilus* does the imago appear prior to that month, and in several the date is much later (*Anabolia nervosa*—August). This direct immigration is not so surprising as it may appear, since all the larvae are capable of active movement: even *Rhyacophila obliterata* often wanders far from its fixed case, while *Agapetus fuscipes*, a very delicate form which makes a completely floored pebble case of relatively enormous weight, can move actively about the Laboratory aquarium carrying its case with it, though it prefers as a rule to cling to a stone.

As an explanation of these remarkable changes in the fauna of the two rivers, any suggestion of a mere seasonal variation must be dismissed, since collections made at corresponding seasons in the preceding years of 1919–1921 had formed the basis of the standard lists of 14 and 9 species only for Rheidol and Ystwyth respectively.

A second possibility suggested itself: that of a down stream migration in consequence of drought (Shelford (12) has shewn that migrations of this and of the up-stream flood type are not confined to Fishes); but this alone is not sufficient to explain the facts. Such a migration has not been known to take place in these particular localities of recent years (notably in the dry season of 1921); from the former distribution of species, very serious changes are seen to be involved, and it is difficult to understand how the newcomers could maintain life in the polluted waters of streams hitherto rendered barren almost from their sources by lead contamination. That life was maintained was evinced by a series of collections made after heavy floods in September of the same year, and in the following spring. During the winter months, when it was of course impossible to compile full lists, as many as 11 of the 15 new species in the Rheidol and 12 of the 17 in the Ystwyth were found to be represented, while in March and April of 1923 the numbers were found to be complete, with the following additions:

Rheidol (Pen y Bont), four species:

Corixa limitata,
Agabus bipustulatus,
Limnebius truncatellus,
Chironomus plumosus;

Ystwyth (Rhyd y Felin), four species:

Planaria alpina,
Velia currens,
Anabolia nervosa,
Deronectes latus;

shewing that the process of repopulation was being continued.

Since paucity of fauna in the area had been consistently related to lead pollution, it was conjectured that the long continued drought, by reducing the amount of surface drainage over the mine heaps, might have produced a diminution in the lead content of the lower rivers. Samples were accordingly taken (in June, 1922), and subjected to quantitative analysis, which gave results as follows:

Locality	Lead in water sample	Lead in grit sample
Pen y Bont (L. Rheidol)	None	0·085 %
Rhyd y Felin (L. Ystwyth)	None	0·01 %

These figures give definite support to the original contention regarding the importance of lead salts in diffusible form.

Probably the drought conditions were not alone responsible for the reduction in lead content of this kind: a contributory cause was no doubt the complete cessation of the last of the local lead mining and washing operations. Until quite recently a few lead mines re-opened during the war had been active, but the last of these affecting the Rheidol (at Rhiwfron and at Melindwr, Goginan) ceased working in 1920 and 1921 respectively, while near the Ystwyth there remained only one centre of activity at Grogwynion, where machinery formerly used for crushing lead ore was now employed upon zinc alone. Doubtless the establishment of the drought soon after the complete cessation of lead washing operations was responsible for the sudden diminution in lead content.

In view of the remarkable reduction in diffusible lead content at the time of the drought, it was decided to investigate the variation in quantity from time to time, taking samples under different weather conditions, mainly at Pen y Bont. Rainfall records are appended, for comparison with the table which follows:

Locality	Date	Diffusible lead content
Pen y Bont (L. Rheidol)	Oct. 24, 1922	0
	Nov. 2 „	0
	„ 7 „	0·05 mgr. per l
	„ 8 „	0·05 „
	„ 17 „	0
	„ 27 „	0
	Dec. 14 „	0
	Jan. 13, 1923	0
	Feb. 2 „	0·1 mgr. per l
	„ 10 „	0·1 „ „
	„ 28 „	0·1 „ „
	Mar. 16 „	0·1 „ „

The diffusible lead content is seen to be very variable in quantity, and, in fact, not present at all in appreciable amount except at times of flood; even then, the figure is low as compared with the old estimates, which ranged from 0·2 to 0·5 milligrammes per litre.

Two samples were taken from the Ystwyth at Rhyd y Felin, on October 24th and November 17th respectively: neither gave any indication of lead in the filtrate.

Samples from streams of "unpolluted" type B, also gave no result from tests of the filtrates; these were taken as follows:

Llangorwen (L. Clarach): November 19, December 15, 1922, January 31, and February 3, 1923.

Bow Street Brook: December 15, 1922.

Capel Dewi Brook: November 10, 1922.

A sample from under a lead mine at Nant Silo (Upper Clarach), on March 5th, 1923, had a lead content of 0·05 mgr. per litre in the filtrate, and a similar figure must be recorded for a sample from under the Rhiw-fron (Rheidol) workings, taken on March 9th.

There has undoubtedly been an improvement in the condition of the lead-polluted waters, which has been in part maintained in spite of very heavy rains during the latter half of December and the whole of January and February, 1923. The parallel improvement in fauna of Lower Rheidol and Ystwyth has also been maintained, and this in full, as mentioned above.

A collection made some miles higher up the Rheidol, at Rhiw-fron, on March 9th, 1923, included the following species, all of which were abundantly represented:

Dendrocoelum lacteum,
Gerris paludum,
Velia currens,
Chloeon simile,
Chloroperla grammatica,
Plectrocnemia conspersa,
Hydroporus rivalis,
Tanypus nebulosus,
Simulium latipes.

This record, from a locality which formerly was practically devoid of animal life, together with the fact of the establishment of much new growth of filamentous green Algae on the stones of the river bed, shews that the improvement is not confined to the lowest course of the river, but extends up into the former mining area.

The general conclusion drawn from observation of the changed conditions is that the presence of a diffusible lead content varying up to 0·1 mgr. per litre, together with lead grit in the river sludge, is no barrier to the establishment of a fairly rich invertebrate fauna. No Mollusca have as yet made their appearance in the streams in question, and no Fishes, with the exception of occasional trout, which stray in from the tributaries, to meet an untimely end, and of sewin, which sometimes find temporary shelter in the lower pools, returning ultimately to the sea. The general improvement in flora and fauna has, however, been so marked and rapid as to lead one to speculate on the possibilities of complete rehabilitation, such as tradition asserts to have occurred at least once in these streams, during the period prior to the revival of mining activities in the early nineteenth century. It may be that the restoration of the invertebrate fauna is an initial step in the process of rehabilitation, but it is unwise either to prophesy completion or to speculate on a method of controlling the pollution due to lead-mining in general without much more definite knowledge of the actual nature and working of the destructive agent. The circumstantial evidence derived from local study and reported in these pages, while strongly favouring the importance of dissolved lead-compounds as the active agent, is not in itself conclusive: its strongest claim is to furnish data which may be used as guiding principles in the conduct of a method of direct experimentation in field and laboratory. Such a method is now in use, and gives reasonable promise of a series of results from which conclusions of a definite character may be drawn, and which may be reported at no far distant date.

SUMMARY.

1. The relative poverty of the fauna and flora of certain streams in the Aberystwyth district can only be related to their pollution by lead mining and lead washing operations.

2. Such operations affect the rivers by the discharge into their waters of galena particles in all stages of division, and also by the formation of lead salts in diffusible form through chemical interaction with the natural water.

3. Only a single case is known of the clogging of fish-gills by

adhering particles of galena-grit: there is no definite evidence that this is a usual cause of death.

4. Statistics locally collected shew, however, that such lead grit is present in practically all the streams of the neighbourhood, particularly in the Lower Clarach, where a rich fauna, including Fishes, subsists.

5. The correlation of fauna lists with tables of analyses of water samples shews that poverty of fauna, with absence of fishes in particular, always accompanies the presence of lead salts in diffusible form.

6. The cessation of the last of the lead mining and washing operations affecting the Rheidol and Ystwyth was followed by a marked and rapid increase in the flora and fauna of the rivers, which was accelerated by the spring drought of 1922. At this season, the amount of lead in diffusible form in these waters was, if any, too small to be estimated, while the proportion of lead grit remained high.

7. Observations made since May, 1922, shew that the original proportion of lead in diffusible form has not been restored, while the flora and fauna have continued to increase.

8. The general inference is that, while the presence of lead grit is no doubt in some cases directly harmful to Fishes, the general fauna of the rivers is undoubtedly impoverished by the presence of lead salts in diffusible form.

More conclusive evidence is hoped for as the result of the employment of an experimental method based on the data reported above.

RAINFALL STATISTICS.

A. Records from Castle Point Station, Aberystwyth. Prior to June, 1922.

Annual rainfall for	1911	32·24″	1916	30·69″
” ”	1912	36·71	1917	33·62
” ”	1913	36·17	1918	36·16
” ”	1914	32·25	1919	32·87
” ”	1915	25·30	1920	42·10

Average annual rainfall 1911–1920 33·81″

Annual rainfall for 1921 26·58″ (7·23″ below average)

Rainfall for Jan. to June, 1922 14·03″:

[Jan.	3·76″	Mar.	2·67″	*May	1·38″
Feb.	3·76	*Apr.	1·76	*June	1·00]

* Directly precedent to drought conditions which accompanied the changes in fauna of L. Rheidol and L. Ystwyth.

B. Records from Castle Point, Aberystwyth, and from Devil's Bridge, to accompany tables of water analysis (the rainfall at Devil's Bridge is important as influencing the condition of streams rising in the High Plateau).

	October 1922		November 1922	
	Devil's Bridge	Castle Point	Devil's Bridge	Castle Point
1	0·01	—	0·34	0·08
2	—	—	0·02	—
3	0·05	0·02	0·05	0·13
4	0·26	0·23	0·04	—
5	0·52	0·10	0·56	0·30
6	—	—	0·63	0·33
7	—	—	0·02	0·07
8	—	—	0·10	0·06
9	—	—	0·01	0·01
10	—	—	0·30	0·12
11	—	—	—	—
12	—	—	—	—
13	—	—	0·01	—
14	—	—	0·01	—
15	—	—	—	—
16	—	—	0·01	—
17	—	—	—	—
18	—	—	—	—
19	0·02	—	—	—
20	—	—	0·03	0·03
21	—	—	—	—
22	—	—	0·01	0·10
23	—	—	0·02	—
24	—	—	—	—
25	—	—	—	0·01
26	—	0·01	0·06	0·01
27	—	—	0·13	0·12
28	—	—	—	0·01
29	—	—	0·01	—
30	—	0·01	0·17	0·08
31	0·77	0·26	—	—
Total ...	1·63	0·63	2·53	1·46

	December 1922		January 1923	
	Devil's Bridge	Castle Point	Devil's Bridge	Castle Point
1	0·19	0·02	0·29	0·18
2	0·01	0·01	0·55	0·16
3	0·01	0·01	—	0·01
4	0·08	0·02	0·12	0·01
5	0·08	—	0·08	0·40
6	—	—	0·67	0·63
7	0·13	0·06	1·84	0·18
8	0·13	0·03	0·52	0·10
9	—	—	0·31	0·01
10	—	—	0·04	0·02
11	0·01	0·02	0·04	—
12	0·07	—	—	—
13	0·04	—	0·26	0·12
14	0·70	0·72	0·04	—
15	0·07	0·03	0·23	0·03
16	0·13	0·08	0·02	—
17	0·07	0·04	0·11	0·10
18	0·13	0·12	0·08	—
19	0·49	0·44	0·64	0·25
20	0·20	0·02	0·07	0·02
21	0·63	0·24	0·21	0·04
22	0·73	0·39	0·02	0·03
23	0·33	0·06	0·02	—
24	0·46	0·13	—	—
25	0·29	0·05	0·05	0·02
26	0·36	0·43	0·02	0·01
27	0·36	0·12	0·05	—
28	0·06	0·20	0·78	0·27
29	0·48	0·24	0·90	0·23
30	0·16	0·22	0·40	0·08
31	0·01	0·01	0·49	0·21
Total ...	6·41	3·71	8·85	3·11

	February 1923		March 1923	
	Devil's Bridge	Castle Point	Devil's Bridge	Castle Point
1	1·01	0·14	0·18	0·10
2	0·02	0·14	0·05	0·03
3	—	0·01	0·26	0·05
4	—	—	0·06	0·07
5	0·04	0·01	0·06	0·03
6	0·75	0·46	0·31	0·17
7	0·86	0·76	0·24	0·04
8	0·08	0·01	0·01	—
9	0·22	0·08	—	—
10	0·32	0·22	—	—
11	0·22	0·06	0·02	—
12	0·57	0·46	0·11	0·02
13	0·52	0·30	0·14	—
14	—	—	—	—
15	0·13	0·06	—	—
16	0·71	0·27	—	—
17	0·63	0·65	—	—
18	0·64	0·16	—	—
19	—	—	—	—
20	0·24	0·06	—	—
21	0·35	0·22	—	—
22	0·30	0·04	—	0·10
23	0·05	—	0·08	0·10
24	0·15	0·09	—	—
25	0·41	0·27	—	—
26	0·87	0·31	0·03	0·01
27	0·41	0·08	0·13	0·02
28	0·60	0·17	0·34	0·18
29	—	—	—	—
30	—	—	0·41	0·27
31	—	—	0·04	0·01
Total ...	10·10	5·03	—	1·20

My grateful acknowledgments are due especially to the following: to Professor T. C. James, of U.C.W. Chemical Department, for assistance and advice in the matter of water analyses; to Mr John Evans, Analyst for the County of Cardigan, for analysis of solid samples; to Professor Fleure, of U.C.W. Geographical Department, for data quoted in the text; to Dr Abraham Thomas, Medical Officer of Health for the Borough of Aberystwyth, and to Mr W. E. Whitehouse, of U.C.W. Geographical Department, for rainfall records supplied; to Mr J. R. le B. Tomlin, and Mr F. W. Edwards, both of the British Museum of Natural History, Mr F. Balfour-Browne, of Cambridge, Mr J. E. Collin, of Newmarket, Rev. Hilderic Friend, of Birmingham University, Rev. J. E. Hull of Belford, and Mr C. D. Soar, for assistance in the identification of species; to Professor R. D. Laurie, of U.C.W. Zoological Department, for continued sympathy and advice; to the Department of Scientific and Industrial Research, under whose auspices the work has been carried on during the past twelve months.

LIST OF WORKS OF REFERENCE.

Numbered references occur in the text.

(1) (*a*) Pollution of Rivers Act, 1876.
  (*b*) Fisheries Act, 1891.
  (*c*) Salmon and Freshwater Fisheries Bill, 1922.

(2) ROBERT HUNT (1848). Notices of the History of Lead-Mines. *Mem. Geol. Soc.*

(3) JONES, O. T. (1922). Special Reports on the Mineral Resources of Great Britain. *Mem. Geol. Survey.*

(4) CLARK, W. M. and LUBS, H. A. (1920). *The Determination of Hydrogen Ions.* Baltimore.

(5) CARPENTER, K. E. (1922). The Clarach Valley: a Preliminary Study of the Problem of Lead-Pollution. *Aberystwyth Studies.*

(6) THRESH, J. C. (1921 and 1922). "Notes on the so-called Action of Water on Lead," and "The Action of Natural Waters on Lead." *The Analyst.*

(7) HOGG, T. W. (1895). Immunity of Some Low Forms of Life from Lead-Poisoning. *Chemical News.*

(8) (*a*) OLIVER, T. (1914). *Lead-Poisoning.* London.
  (*b*) LEGGE, T. M. and GOADBY, K. W. (1912). Lead-poisoning and Lead-absorption. *International Medical Monographs.*

(9) *Fourth Report of the Royal Commission on Pollution of Rivers in Scotland,* II, Evidence.

(10) *Salmon and Trout Mag.* No. 28, Jan. 1922.

(11) CLOWES, F. and COLMAN, J. B. *Text-Book of Quantitative Analysis.*

(12) SHELFORD, V. *Animal Communities in Temperate America.* Chicago Univ. Press.

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APPLES ATTACKED BY THE LARVAE OF THE DOCK SAWFLY (*AMETASTEGIA* (*TAXONUS*) *GLABRATA*, FALLÉN)

By F. R. PETHERBRIDGE, M.A.

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(With 3 Text-figures.)

In October 1922 specimens of apples each with one or more small holes bored into them were received from a fruit farm in East Norfolk.

I had not previously seen damage similar to this and was surprised to find on cutting open the apples greenish sawfly larvae which resembled a number of leaf-eating forms. On breeding the sawfly we found it to be *Ametastegia* (*Taxonus*) *glabrata*, Fallén.

The grower stated that about 1 per cent. of the variety Lord Derby and also a few Bramley's Seedlings were attacked. The apples had previously been picked from the trees and stored in a large barn.

Historical and Distribution.

This sawfly was described in Sweden by Fallén in 1808. Cameron says: "It is a very common and universally distributed species." It was first recorded as a pest of apples by Fletcher in Canada in 1903, and several investigators have since recorded damage similar to the above in America and Canada, where an injury of 10 per cent. of the crop is the maximum recorded.

A full description of the life history in America and the damage caused by this pest is given by E. J. Newcomer in *Bulletin* 265, U.S. Department of Agriculture. It has previously been recorded as boring into apples in this country by Theobald in 1917 at Allington, Kent (*Monthly Reports to the Ministry of Agriculture*). The larvae are usually found feeding on the various species of *Rumex* and *Polygonum*, but have been recorded on other plants such as sugar beet, loosestrife (*Lythium salicaria*) and *Viola tricolor*. I have only found it feeding on species of *Rumex*.

NATURE OF INJURY. (See fig. 1.)

The injury to the apples consists of small holes about 2 mm. in diameter, the edges of which are brownish in colour, and also of burrows of similar diameter and of varying lengths. Frass is usually present near the holes, which are present anywhere on the surface of the apple, and apparently as frequent in one part as another. Newcomer says: "These holes may occur anywhere on the surface, but are more numerous about the calyx and stem end, or at a point where the apple touches a leaf or another apple, since it is easier for the larvae to obtain a foothold here."

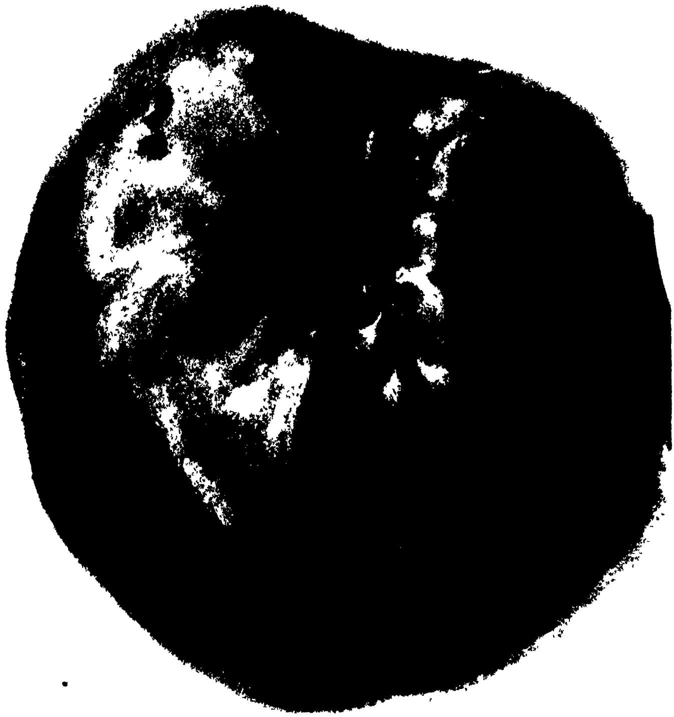

Fig. 1 *a*. Lord Derby apple showing holes and frass made by *Ametastegia glabrata*.

The burrows vary from less than a quarter of an inch to about two inches long. They also vary considerably in shape, some being straight and others curved. A few of them reached to the core and some were semi-circular with an entrance and an exit hole. The number of burrows in individual apples varied from one to six and a large percentage of the burrowed apples contained no larvae. Some of the burrows were larger at the ends and here the larvae were found facing the holes and here they eventually made their pupal cases.

The damage may easily be distinguished from that of the Codlin Moth caterpillars by the fact that the burrows rarely reach the core. They do not bore into the eye nor do they eat the core as the codlin moth caterpillars do.

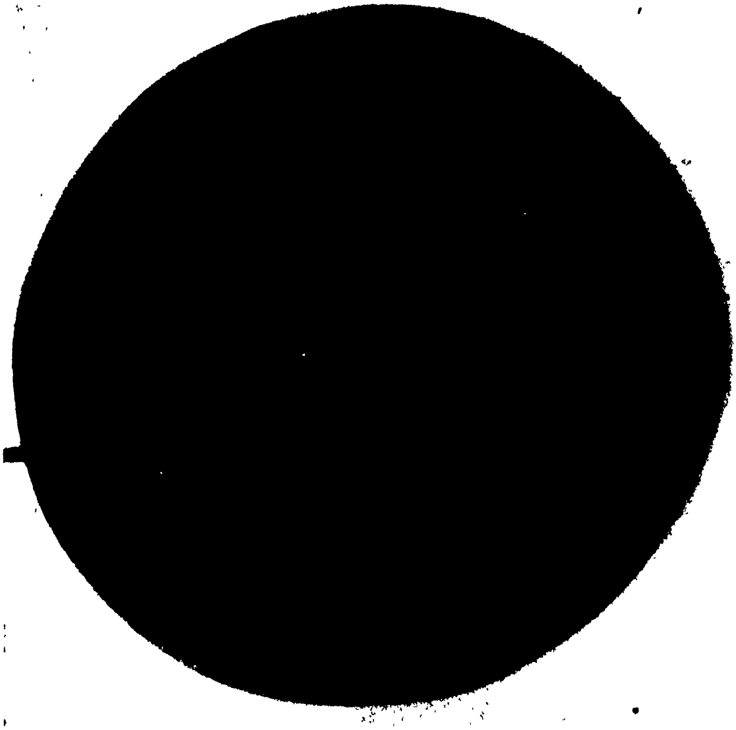

Fig. 1 *b*. Bramley seedling apple showing hole made by *Ametastegia glabrata*.

The apple sawfly (*Hoplocampa*) larva is earlier in the season, makes a much larger burrow, and does much more damage.

The holes made by the dock sawfly larvae naturally provide easy access for the spores of various fungi and, as expected, many of the apples began to rot round the holes. The tissue round the pupa case became very dry and did not suffer from wet rot like the tissue along the beginning of the burrow. Some of the burrowed apples kept until the end of March without showing signs of rot.

Hibernation. For fear that the larvae might be drowned by the juice of the rotting apples most of them were removed and placed in the ends of old raspberry canes, from which the pith had been scooped out, as this was thought to approximate their normal method of hibernation. In some cases the dried apple tissue surrounding the larva was removed with it. They were kept under the roof where the temperature approximates that of natural conditions.

The larvae remained all through the winter without pupating. The first pupation took place on March 5th, but this was probably abnormally early, as on April 5th an ichneumon parasite emerged through a round hole at the head end. This was identified by Dr Otto Schmiedekneckt as *Mesoleius armillatorius*, Gravenhorst, which is not uncommon in Great Britain.

The normal larvae remained as such much longer, the first pupa was not formed until April 28th and from this I obtained a female sawfly on May 8th. Most of the remainder pupated in May, but one larva did not pupate until after the middle of June. The sawflies come out in May and June.

LIFE HISTORY.

Cameron says: "there are two broods of larvae, the first in June and July and the second from August to September, and this last generation seems to be the larger of the two."

This agrees with my observations, as I found adult larvae only in July and early August and at the end of September and beginning of October.

The spring generation of flies lay their eggs mostly in June in the leaves of docks and allied species. The larvae which hatch feed on the leaves eating irregular holes until fully grown, when they leave their host to pupate in the pithy stems of various plants. The second generation also lay their eggs on docks and allied plants; in August, and usually when fully fed at the end of September or early October, they migrate to the pithy stems of various plants or, as shown above, bore into the fruit of apples to hibernate and then to pupate in the spring.

In America, Newcomer says: "there are four generations which overlap and become somewhat confused."

Egg. The egg is kidney-shaped about 0·8 mm. by 0·6 mm. and white in colour.

Larvae. At first the larva is light in colour with the alimentary canal showing through. Later on the dorsum becomes bluish green with an almost black colour on the tops of the wrinkles, and the rest of the body of a dirty yellow colour. At the last moult the dorsum becomes apple green in colour with the sides shading from light green at the sides to yellow on the sternum.

LARVA FOUND IN APPLES. (See fig. 2.)

About half an inch or less in length (specimens found October 4th, 1923, on docks measured $\frac{11}{16}$th of an inch).

Colour. Dorsum to line of spiracles apple green, sides of sternum paler shading from very light green at the sides to yellowish green on the sternum.

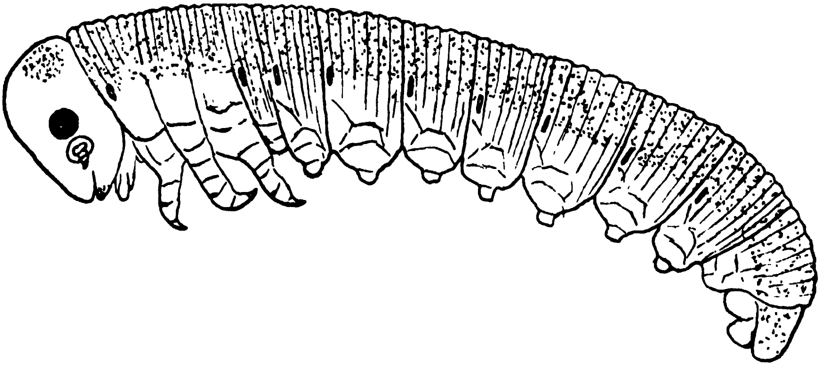

Fig. 2. Larva of *Ametastegia glabrata* from an attacked apple.

Legs. Five segments yellowish, dark brown line across the apex of the middle and a less distinct line across the base of the segment. Brownish curved claw at apex, a few spines present.

Prolegs. Yellowish green; 8 pairs on abdominal segments 2–8 and the last abdominal segment.

Spiracles. Four times as long as broad. Black, surrounded by smooth patch of skin. Occur just at junction of the dark green with the lighter colour. Nine in number, on first thoracic segment and first eight abdominal. Length of thoracic spiracle 170μ by 35–39μ. Abdominal spiracle 102–119μ long.

Body. Cuticle much wrinkled and finely shagreened.

Head. Declined backward, very light shade of brown with dark olive patch on the apex not extending to first thoracic segment.

Eyes. Black, raised, with a black ring round them, situated in pale-coloured part of head.

Antennae. Just below the eyes with a broad basal segment and four small joints darker in colour.

Mandibles. Yellowish at base, with three large teeth dark brown in colour.

Pupa. At first bright green on the dorsal region, with the rest of the body, head, legs and antennae pale yellowish green. Eye large and reddish brown. Before the adult emerges the pupa becomes almost black.

Adult. As Cameron says, its reddish legs, black posterior tarsi and *coppery, violet* body distinguishes it from the other species. He says it is very common and universally distributed.

EXPERIMENTAL.

Experiments were carried out to show that the damage was due to these larvae, and for this purpose two larvae found feeding on docks in Norfolk were placed in a bell jar with four apples on September 23rd, 1923. At first the larvae fed on the dock leaves, but when these were dried up they began to bore into the apples. On September 28th they had made 8 holes, on October 3rd 13 holes and on October 9th they had made 16 holes and were both present in the apples.

Previous to this Miss Chawner succeeded in making the first brood bore into an apple. On August 2nd, 1923, she wrote as follows: "I succeeded in rearing 4 larvae from virgin eggs laid by the fly which you sent me. I experimented as follows: To one I gave pith, into which it bored. To two I gave pith and a sound young apple to choose between—both preferred the pith. To the remaining one I gave only a sound apple; it bored into it with perfect ease. I may add that all four larvae have produced male imagines as is customary with unfertilized eggs laid by this species."

ECONOMICS.

As the larvae of this pest feeds almost entirely on docks and polygonums and does not feed on the leaves of the apple, it is not likely to become a serious pest. In the orchard where the apples were injured docks were fairly plentiful in 1922; but were kept down in 1923 with the result that no damage was done and only a few larvae were found in the hedges. Even in orchards where host plants are growing the larvae would probably be prevented from ascending the trees by suitable banding.

I am indebted to Mr J. W. Cowland for assisting me with this work.

SUMMARY.

The larva of the dock sawfly, *Ametastegia glabrata*, Fallén, which is a leaf feeder usually found on docks and polygonums, injured apples in East Norfolk by boring small holes into the flesh in which to pupate. They made from one to six holes in each fruit. They were kept in check by keeping the orchard clean.

BIBLIOGRAPHY.

CAMERON, P. (1882). *Monograph of British Phytophagous Hymenoptera*, I, 204.

FLETCHER, J. (1903). *U.S. Dept. Agr. Ent. Bul.* XL, 78–83.

NEWCOMER, E. J. (Dec. 1916). *U.S. Dept. Agr. Bulletin*, No. 265. This gives a detailed account of the pest and a bibliography.

(*Received October 20th*, 1923.)

POTATO LEAF-ROLL AND DEGENERATION IN YIELD

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THE now generally recognised economic importance of the 'virus' diseases of the potato is due, no doubt, to the belief that they are mainly responsible for the 'running out' or degeneration in yield, so noticeable in most potato growing districts when 'seed' is saved for planting in two or more successive seasons. At present, whilst there is ample evidence that virus diseases do reduce the yield to a considerable extent there is no sound evidence that degeneration may be due to other causes, such as senescence or enervating conditions of growth.

In the absence of definite proof that diseases of the virus type are due to the action of specific organisms, they must still be described in terms of the symptoms produced in infected plants. The usefulness of this method of segregating diseases is limited by the fact that both external conditions and varietal characteristics greatly modify the symptoms observed. It is, therefore, not yet possible to say whether diseases described as simple (as distinct from composite) in one country (16, 18), occur in another in which climate, soil characters, cultural operations and varieties grown, may be very different. These difficulties are less marked in the case of leaf-roll, for although the symptoms may vary considerably with the variety affected and to some extent with external conditions, there is little doubt, in most cases, as to the identity of the disease in its second year or secondary form. In this secondary form leaf-roll can usually be diagnosed with ease by the dwarfing of the plant accompanied by the development of a rigid habit and stiff, leathery, pale green, upwardly-rolled leaves. Frequently all leaves are affected but not uncommonly rolling only takes place in the lower leaves. Quanjer's primary symptoms (13) have rarely been observed with certainty in any variety commonly grown in N. Wales, and never in the variety Arran Comrade used in the present investigation. The development of colour in affected leaves and the persistence of the 'mother' tubers—although common—are not constant symptoms of leaf-roll. It is often stated (19) that leaf-roll increases the proportion of 'seed' and 'chats' in the crop; that this is not invariably the case will be seen later. In the writer's opinion, both the occurrence of necrotic elements in the phloem and of excess starch in the leaves—as reported by Quanjer (13) and Murphy (9, 11) respectively—are reliable aids in diagnosing leaf-roll. No method for the diagnosis of leaf-roll in the seed-tuber has been published. This would greatly increase the possibility of controlling the disease, and that such an advance is not too much to hope for is shown by the considerable difference in dry matter and nitrogen content in tubers from rolled plants when compared with tubers from healthy plants grown under the same conditions; facts which have been repeatedly established in the course of work in progress by Mr W. McLean of this College, in collaboration with the writer.

Present Work. The work described below, on which the writer was engaged during the years 1921, '22 and '23, was concerned entirely with the economic aspect of leaf-roll in North Wales, and not with the aetiology of the disease. The variety used throughout was Arran Comrade and both diseased and healthy stocks were selected and sent to the writer by Mr A. D. Cotton, late Mycologist to the Ministry of Agriculture. Similar stocks were sent to other centres and the yields obtained in 1921 from both kinds of 'seed' were published by Cotton (6, 7).

During the present work, large differences in yield between healthy and rolled plants have been taken as a measure of the degree of infection. In arriving at conclusions, however, it has not been forgotten that other factors may influence the yield to a considerable extent; such factors, for instance, as origin of 'seed,' slight variations in methods of storage, time of planting or cultural operations, and differences in soil, have been eliminated as far as possible.

Some preliminary experiments in 1921 may be disposed of briefly.

(1) *Communicability of leaf-roll by grafting.* Infection was obtained by joining the halves of a diseased and a healthy tuber together, cementing the edges with grafting wax to prevent undue evaporation, and carefully excising the 'eyes' from the diseased half. The composite tuber produced diseased shoots from the originally healthy half, as did also the rolled-half control, whereas the healthy-half control produced a normally healthy plant. This confirms Quanjer's statement (14) as to communicability of the disease in this way.

(2) *No control of leaf-roll by immersing tuber in corrosive sublimate.* Forty tubers from diseased stock were immersed in 1/1000 mercuric chloride for an hour and a quarter; the solution being allowed to cool

from 37° C. They were then rinsed in tap-water, left overnight to dry and planted alongside untreated tubers. One or two plants were apparently healthy but no more than in the controls. Blodgett has since (2) tried the effect of hot water, as well as mercuric chloride but finds that in both cases all potatoes surviving the treatment were affected with leaf-roll.

(3) *Effect of cutting diseased tubers before planting.* The well-known fact that some varieties, *e.g.* Majestic, do not 'cut' well suggested an experiment to see if this also applied to leaf-roll tubers. Each of forty half-tubers planted produced a plant and the yield as compared with that from the same number of cut healthy tubers closely approximated to the results in other trials in which the tubers were not cut.

Loss in cropping power due to leaf-roll. An attempt has been made during the last three years to determine the loss of cropping power due to leaf-roll, and to ascertain whether the effects are cumulative under the conditions of N. Wales.

In all the results reported below comparisons are made between the yields of diseased and healthy stocks growing in adjacent rows.

1921. The average yield from 17 rows of leaf-roll plants was 32·1 lbs. per row, whereas 8 rows of healthy plants gave an average yield of 72·6 lbs. per row of the same length. If the yield of the healthy rows is taken to = 100, that of the diseased rows = 44·2; showing a loss in yield of 55·8 per cent.

1922. The average yield from 12 rows of the progeny from the 1921 leaf-roll plants was 48·9 lbs. per row and that from 6 rows of new Scotch 'seed' was 89·9 lbs. Again taking the latter yield as = 100, the yield from the diseased stock was 54·4, *i.e.* a loss of 45·6 per cent.

1923. One row each of new Scotch 'seed' and the progeny of the original diseased stock were planted in adjacent rows in different parts of the same field. As was expected, the yields varied in the different plots, doubtless owing to soil differences or other variable factors. The losses in four such plots were respectively 55·2, 46·6, 45·2 and 63·4 per cent.

It does not appear from these results that the effects of leaf-roll are cumulative under the conditions obtaining in N. Wales. This opinion is strengthened by a comparison of the losses in yield in stocks previously healthy but infected with leaf-roll in 1922 and grown in adjacent rows in the four plots mentioned above in 1923; these show losses respectively of 50, 44·8, 60·9 and 57·4 per cent.

Similar conditions apparently are found at Craibstone in the North of Scotland, for in the variety Kerr's Pink the loss due to leaf-roll was 73·2 per cent. in 1920 and 73·9 per cent. in 1921 (20). In a letter to the present writer, Mr W. M. Findlay, of Craibstone, states that affected plants show a progressive loss in cropping power during the first year or two, after which the yield is either maintained or even improved as the plants become adapted to their changed conditions.

The opinion is so strongly held by pathologists that leaf-roll is cumulative in its effects that an attempt has been made to get definite evidence on this point. The only example the writer has been able to find is at Leeds, where, using the same stocks as at Bangor, losses of 39·7 and 60 per cent. were obtained in 1921 and 1922 respectively[1].

It should be observed that the increased loss at Leeds occurred in the year in which a decreased loss was recorded at Bangor. There is some evidence, therefore, that the disease becomes intensified in the progeny in some localities, *e.g.* Leeds, whereas in others a progressive deterioration or 'running out' is not likely to occur.

That cultural operations as well as climate may affect the yield from leaf-roll plants was shown in 1921 by leaving a row, in which healthy and diseased tubers were planted alternately, entirely uncultivated from planting to lifting time; the yield of the rolled stock showing a loss of 80·3 per cent. as compared with that from the healthy plants. Again, in 1923 two rows of healthy and diseased plants were left after 'earthing up' without further attention. Weeds over-ran the rows and a loss in yield of 70·1 per cent. was recorded.

Effect of leaf-roll on the size of tubers produced. It is usually accepted that leaf-roll plants produce only a small number of 'seed' size or small tubers in place of a normally healthy crop of tubers varying in size from 'ware' to 'chats' (19). To test this view the number of 'ware,' 'seed,' and 'chats,' was obtained in 1922 and 1923 from alternate rows of diseased and healthy plants. By ware and seed are to be understood tubers which do not pass through a 1½″ and 1″ square mesh respectively. In 1923 the average weight of 133 diseased ware tubers was = 4·8 ounces; that of 217 healthy ware being 4·4 oz. Similarly, the average weight of 176 diseased seed and of 517 healthy seed was 1·7 oz. and 1·8 oz. respectively.

The results obtained were as follows:

In 1922 the average of ten rows of rolled plants showed that seed-size and small tubers together represented 73·8 per cent. of the total number of tubers produced; the figures for six rows of new Scotch stock and once-grown healthy being 74·0 and 72·4 per cent. respectively. Using the same method in 1923, the figures obtained were 60·6 per cent. for four rows of plants rolled in 1920, '21, '22, and '23; 70·3 per cent. for four rows of new healthy Scotch stock, and 56·4 per cent. for two rows of once-grown healthy plants.

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[1] The figures are based upon information received from Mr F. T. Bennett who carried out the trial at Leeds.

In the same way the proportion of seed-size and small to the total *weight* of tubers produced was determined in 1921, '22, and '23; with the following results:

1921. Rolled plants = 78·4 per cent.; new Scotch = 75·3 per cent.

1922. Rolled plants = 46·8 per cent.; new Scotch = 49·7 per cent.; once-grown = 38·8 per cent.

1923. Rolled plants = 30 per cent.; new Scotch = 41·1 per cent.; once-grown = 29·9 per cent.

It appears from these results that it is an error to suppose leaf-roll increases the proportion of 'seed' and small tubers in the crop in this variety. On the other hand they support the general opinion amongst farmers in N. Wales that new Scotch seed usually gives a larger number of the smaller tubers than does once-grown seed. The results also suggest that weather conditions will greatly influence the size of tubers obtained, for whereas 1921 was very unsuitable for potato culture owing to the drought and heat, ample rain fell in the summers of 1922 and 1923 to allow of continued tuber growth.

Effect of leaf-roll on the total number of tubers produced. The reduction in the total number of tubers produced as a result of leaf-roll infection was ascertained in 1922 and 1923 by comparing the average yield per plant of diseased and new Scotch stock.

1922. Twelve rows of diseased stock gave averages of 9·4, 8·3, 7·0, 8·9, 8·5, 7·2, 7·2, 7·4, 8·7, 8·4, 8·4, and 6·3 per plant; an average throughout of 7·9 tubers per plant.

Six rows of new Scotch seed gave 16·4, 16·5, 16·0, 14·3, 13·2 and 13·6; an average of 15 tubers per plant. The reduction in total number was, therefore, 47·3 per cent. in 1922.

1923. Eight rows of diseased stock gave 6·4, 5·7, 4·6, 5·8, 8·3, 6·5, 5·9, and 6·6; an average of 6·2 tubers per plant.

Two rows of new Scotch seed growing amongst the above rows gave 18·6 and 13·2; an average of 15·9 tubers per plant. The reduction in total number was, therefore, 61 per cent. in 1923.

Transference of leaf-roll under field and garden conditions in 1921. With the object of determining the conditions under which transference of leaf-roll takes place plants were exposed to different chances of infection in 1921 in the following way:

(*a*) A row of the original healthy 'seed' from Scotland was planted in a field alongside new and once-grown stocks of a large number of varieties. These plants were, therefore, exposed to the ordinary chances of infection under farm conditions.

(*b*) Two rows of the same healthy 'seed' as in (*a*) were planted between four rows of rolled plants. In this way each of the two rows was exposed to infection from one side, both above and below ground. This plot was laid down in triplicate.

(*c*) Two rows in which diseased and healthy sets, placed alternately one foot apart, were planted between diseased rows so that the healthy plants were exposed to infection from all four sides both above and below ground; this was duplicated.

(*d*) One row of healthy and diseased sets, placed alternately one foot apart, were planted between diseased rows. Large roofing 'slates' were driven into the ground to a depth of about 4″ below the mother tubers, with the object of preventing the intermingling of the roots of adjacent plants. The healthy plants were therefore exposed to infection from all four sides above ground, whereas below ground they were partially protected from infection from neighbouring plants in the row.

Seed was saved from each of these rows and planted in 1922 with the following results:

(*a*) *Field-grown in* 1921. Average of six rows = 101·3 lbs.; average number of tubers per plant = 17·3.

(*b*) *Exposed to infection from one side in* 1921. Average of two rows = 105·75 lbs.; average number of tubers per plant = 17·65.

(*c*) *Exposed to infection from all four sides in* 1921. Average of two rows = 54·25 lbs.; average number of tubers per plant = 7·8.

(*d*) *Exposed to infection from all four sides but roots of healthy plants partially isolated by 'slates.'* Average of two rows = 72·1 lbs.; average number of tubers per plant = 10·85.

(*e*) *Plants rolled in* 1920, '21, '22. Average of two rows = 52·1 lbs.; average number of tubers per plant = 8·85.

No aerial insects were seen feeding on the potatoes although a careful search was frequently made during the summer of 1921.

These results justify the conclusion that no infection occurred under the conditions of (*a*) and (*b*). In both cases the foliage in 1922 was apparently quite healthy.

In Plot (*c*) maximum infection appeared to have taken place for the loss in yield was fully as great as in the stock known to have been diseased for three years.

In Plot (*d*) where the roots of healthy plants were partially isolated there was a reduction of only 29 per cent. as compared with the healthy (field-grown 1921) stock. This reduced loss cannot be explained on the ground that some plants escaped infection since all the progeny in 1922 produced plants which were distinctly rolled, although the disease was not so marked as in the case of plants rolled since 1920.

Transmission of leaf-roll only occurred between plants in the same row; the disease did not spread across the rows although the foliage was in close contact during a great part of the growing season. So far as the writer can see, the only explanation which covers the facts is the supposition that transmission took place through the soil. Since all efforts by previous workers to effect transference of leaf-roll or mosaic by mere contact of uninjured roots have failed, it is perhaps most probable that soil fauna were responsible for the transference through the soil; if this were so it might explain why transmission was readily effected through the loose-worked soil of the row and not across the consolidated soil between the rows.

Some positive results obtained by Quanjer in his earlier work (13) led him to believe that the 'virus' can exist saprophytically in the soil, but his later work has convinced him that this is an error (14, 15, 16)—thus bringing leaf-roll into line with accepted views on other virus diseases of plants. It appears to the writer that this has led to a general disbelief in soil *transmission*—a very different thing. Quanjer showed that in every case where the roots of healthy plants were isolated from diseased ones a healthy progeny resulted, whereas in those cases where the roots were not isolated the progeny was invariably diseased (14, 15). Aphides occur on the potato foliage at Wageningen and no precautions appear to have been taken to protect the plants against aphis attack, yet though the foliage was in close contact, no infection resulted so long as the roots were protected. A similar experiment in which the same conclusion was arrived at has been recorded by Murphy (8). Quanjer (14, 15) and Oortwijn Botjes (3) have explained certain obscure cases of infection as being due to transmission from volunteers or from tubers found at a depth of half a yard which produced no shoots but only tuber-bearing stolons. It is clear that the occurrence of the latter can have no significance unless the possibility of soil transmission is accepted.

Transference of leaf-roll under garden conditions in 1922. In 1922 six rows each of new healthy Scotch seed and of once-grown healthy seed were grown in alternate rows with the original diseased stock. The disease was readily transferred from the leaf-roll rows to the next healthy ones for the progeny of the healthy rows planted in 1923 produced diseased plants and the loss in yield, in most cases, was as great as in the originally infected stock.

During the summer of 1922 the experimental plots were examined for potato aerial insects by the Entomologist at this College, Mr C. L. Walton, who collected the following species on the foliage:

Macrosiphum solanifolii (Ashmead), fairly common.
Aphis solanina (Passerini), a few only.
Chlorita sp., one specimen only.
Sminthurus luteus (*Bourletiella lutea*), a few only.

No potato insects were found in the field on the farm crop.

The conclusive evidence which has been brought forward during the last few years that aerial insects are capable of transmitting leaf-roll to healthy plants (4, 17) leaves one in no doubt that the greater spread of leaf-roll at Bangor in 1922 is to be correlated to some extent with the appearance of insects on the foliage.

Effect of leaf-roll on the plant during the year of infection. It has been shown that healthy plants, exposed in 1921 to infection from diseased plants alternating with them in the same row, were so heavily infected as to produce a crop in the *following* year only equal to that from plants known to have been diseased for three years. During the year of infection (1921), however, the yield was fully as good as that from seed in which no infection had occurred; the former yielding 95 lbs. as compared with 84 lbs. in the two nearest rows of the non-infected stock.

There appear to be three possible explanations of this result:

A. Infection took place through the foliage and the virus passed direct to the developing tubers. This implies that the virus, just extracted from the foliage of a diseased plant, was unable to produce any marked physiological disturbance in the healthy plant during its movement to the tubers, although the same virus in the following year prevented the normal functioning of the elements concerned with translocation of carbohydrates to such an extent that the crop was reduced by 55·8 per cent.

B. Infection took place as in A, but the amount of inoculum was too small to cause appreciable physiological disturbance in traversing the aerial parts of the plant. The effects produced in the following year could then only be explained on the assumption that some development of the virus took place in the tubers during storage. It is probable, however, that if any considerable development occurred during storage no evidence of reduced infection would have been observed when the roots of healthy plants were partially protected from infection.

C. Infection took place through the soil so that possibly the aerial parts of the plants were not infected at all in 1921.

Of these three suggestions the last one appears to be the most probable.

Unfortunately no comparable results were obtained in 1922 when some aerial infection probably took place, for all healthy plants in the trials became infected as was shown in their progeny in 1923. The fact that the loss recorded in 1922 was 10 per cent. or so less than in 1921 *may*, however, be a measure of the degree of infection of the aerial parts of the healthy plants in 1922, rather than of the improvement in the yield from diseased stock. Admittedly, much work will be required to establish the validity or otherwise of this surmise.

Relation of leaf-roll to seed-potato production. So far as our knowledge goes at present seed-potatoes can (or should) only be produced either where no virus diseases exist, or if present, where they spread very slightly. No information is as yet available as to the loss caused by diseases of the mosaic-type, but the results here recorded show the serious effects of obtaining seed slightly affected in Scotland with leaf-roll in 1920.

There is reason to believe that in some localities in the Scottish lowlands potatoes degenerate perhaps as rapidly as in many parts of England, and in some years a high percentage of virus diseases has been recorded in new Scotch seed in N. Wales. It is unlikely that there is any valuable quality inherent in 'seed' of Scottish origin and absent in that obtained elsewhere, for it has been reported that the locality from which seed is obtained is only of importance in so far as it determines the amount of virus disease present in the progeny (12).

In the early literature (1, 5) on degeneration diseases in England insistence is frequently laid on the fact that potatoes from 'cold, mossy' soils or from moorland are not usually subject to 'curl'—a term which doubtless included the disease now known as leaf-roll. Seed from such districts was said to be as good as that from Scotland. In many districts adjoining hill-land in N. Wales it is customary for lowland farmers to secure 'seed' from farms at a higher elevation. Whilst engaged with Mr C. L. Walton in an investigation on the relation between the occurrence of potato insects and virus diseases many areas have been located in which seed of some variety has been saved for many years—in one or two for upwards of 80 years—apparently without any considerable deterioration in yield, and certainly without any obvious symptoms of virus diseases on the foliage. Many of these districts are at no more than 300 feet altitude and one is at sea-level, but all are exposed districts and subject to strong sea winds; potato insects in such localities are very scarce.

The writer is of the opinion that a search throughout England and Wales will reveal many such potentially good seed-potato growing districts.

Grateful acknowledgment is due to Professor R. G. White both for help and criticism, and for the considerable facilities afforded the writer in carrying out this work on the College farm, Aber.

SUMMARY AND CONCLUSIONS.

1. The present communication summarises the first results of work now being carried out at the College farm, Aber, Bangor. It is to be regarded as a first progress report.
2. Quanjer's statement as to communicability of leaf-roll by grafting tubers is confirmed.
3. The unlikelihood that 'seed' sterilisation will be useful as a measure of control is indicated by the failure to effect any control by steeping infected 'seed' in 1/1000 mercuric chloride for an hour and a quarter.
4. Cutting diseased tubers before planting did not affect the yield.
5. The loss in yield due to leaf-roll was 55·8 per cent. in 1921, 45·6 per cent. in 1922 and 52·6 per cent. in 1923.
6. The effects of leaf-roll do not appear to be cumulative under the conditions at Bangor.
7. The proportion of seed-size and small tubers—as determined by relative weights and numbers—to total crop was not increased in the variety Arran Comrade as a result of infection with leaf-roll.
8. The reduction in total number of tubers produced by a leaf-roll crop was = 54·4 per cent. in 1922 and 61 per cent. in 1923.
9. The transference of leaf-roll to healthy plants at Bangor in 1921 and 1922 has been studied. Evidence of soil transmission in 1921 is submitted. In 1922 both aerial and soil transmission is believed to have taken place.
10. Maximum infection in 1921 did not affect the yield in that year.
11. The relation of leaf-roll to seed-potato production is discussed.

LITERATURE.

(1) ATANASOFF, D. (1922). A Study into the Literature on Stipple-Streak and related diseases of the potato. *Mededeelingen van de Landbouwhoogeschool,* Deel 26, verhandeling I.

(2) BLODGETT, F. M. (1923). Time-temperature curves for killing potato tubers by heat treatments. *Phytopathology,* XIII, no. 2.

(3) BOTJES, J. OORTWIJN (1920). Raising Phloem-Necrosis and Mosaic free Potatoes, etc. *Phytopathology,* X, no. 1.

(4) —— (1920). *Die Blattrollkrankheit der Kartoffelpflanze.* Wageningen.

(5) CAMPBELL, DR (1802). 'Curl.' *Communications to the Board of Agriculture,* III, 219.

(6) COTTON, A. D. (1921). The situation with regard to Leaf-Curl and Mosaic in Britain. *Report International Potato Conference,* London.

(7) —— (1922). Potato Leaf-Curl Demonstrations. *Journ. Ministry of Agriculture,* XXVIII, no. 2, 1019.

(8) MURPHY, P. A. (1921). Investigation of Potato Diseases. *Dominion Dept. of Agric. Canada, Div. of Botany, Bull.* 44, 2nd ser.

(9) —— (1921). Some recent work on Leaf-Roll and Mosaic. *Report International Potato Conference,* London.

(10) —— (1922). Leaf-Roll and Mosaic, two important diseases of the Potato. *Journ. Dept. Agric. and Tech. Instr. Ireland,* XXII, no. 3.

(11) —— (1923). On the cause of rolling in potato foliage. *Scientific Proceedings of the Royal Dublin Society,* XVII (N.S.).

(12) PARKER, W. H. (1922). Report on Potato Maturity and Yield Trials, 1921 and 1922. *Journ. National Institute of Agric. Botany,* no. 1.

(13) QUANJER, H. M., et al. (1916). Nature, Mode of dissemination and control of phloem-necrosis (leaf-roll) and related diseases. *Overgedrukt uit Mededeelingen van de Rijks Hoogere Land-bouwschool,* Deel 10, Wageningen.

(14) —— et al. (1919). The Mosaic disease of the Solanaceae, its relation to phloem-necrosis and its effects on Potato Culture. *Mededeelingen van de Landbouwhoogeschool,* Deel 17.

(15) —— (1920). The Mosaic disease of the Solanaceae, its relation to the phloem-necrosis, and its effects upon Potato Culture. *Phytopathology,* X, no. 1.

(16) —— (1921). New Work on Leaf-Curl and Allied Diseases in Holland. *Report International Potato Conference,* London.

(17) SCHULTZ, E. S. and FOLSOM, D. (1921). Leaf-roll, Net-Necrosis, and Spindling Sprout of the Irish Potato. *Journal Agric. Research,* XXI, no. 1.

(18) —— and FOLSOM, D. (1923). Transmission, Variation, and Control of certain Degeneration Diseases of Irish Potatoes. *Journal Agric. Research,* XXV, no. 2.

(19) Anon. (1920). Potato Leaf-Curl. *Journ. Ministry of Agriculture,* XXVII, no. 3, 287.

(20) Anon. (1922). *Guide to Experiments at Craibstone.*

(*Received December 21st,* 1923.)

THE TOXIC ACTION OF COAL GAS UPON PLANTS

II. THE EFFECT OF COAL GAS UPON CORK AND LENTICEL FORMATION

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(With 2 Text-figures.)

INTRODUCTION.

SUMMARIES have recently been given by one of us (7) of the experimental work upon which the conclusion is based that certain toxic effects produced upon plants by traces of coal gas in the atmosphere can be attributed to the presence of unsaturated hydrocarbons. In the case of growing roots and the epicotyls of certain etiolated seedlings it was possible to show that the effect of these unsaturated hydrocarbons could be associated with certain definite structural alterations in a tissue, the endodermis, which depends for its proper functioning upon a deposit of unsaturated fatty acids in its walls at an early stage of its development. Gaseous compounds with unsaturated linkages seem to act by rendering these unsaturated acids more mobile within the walls of the plant, and instead of their retention at the level of the endodermis they migrate towards the surface. Further observation of the phenomena associated with gas poisoning has only tended to confirm this general conclusion, whilst at the same time it suggests that the experimenter is thus provided with a new and valuable means of estimating the significance of the presence of such unsaturated fatty acids within the plant. Hansteen Cranner (3) has recently provided a very considerable amount of experimental evidence that all normal parenchymatous tissues may be expected to include as invariable constituents of their walls, certainly fatty acids, and possibly even phosphatides. It would seem a natural corollary that fatty acids should also form a normal constituent of the wall of meristematic tissues and considerable evidence for this has recently been obtained (Tupper-Carey and Priestley (13)), the iodine number of some of the fatty substance derived from the wall showing further that some of these constituents are certainly unsaturated. As previously suggested, these unsaturated fats may partly be held in the wall as the result of a reversible chemical reaction in which the fatty acid takes part by its unsaturated—CH = CH—linkage, thus retaining its acid properties unaltered. In the presence of a gaseous unsaturated compound such as ethylene substitution of the ethylene linkage for the unsaturated acid renders the latter much more mobile, with its consequent migration, along with the sap stream in the walls, towards the outer surface of the tissue. Whether this explanation proves correct or not, it is hoped to show in this and later papers that the greater mobility of the fatty acids in the wall, in the presence of the unsaturated compounds contained in coal gas, is a phenomenon that permits of ready experimental demonstration, and that this phenomenon plays a very important rôle in most of the toxic effects produced by traces of coal gas upon the plant. In the present paper the effect of coal gas upon the formation of cork tissues is particularly investigated from this standpoint; in later papers it will be shown that similarly the remarkable effects of coal gas upon leaf and flower fall and in the production of epinastic curvature of leaves are associated with the greater mobility of fatty substances in the walls of the plant.

MOVEMENTS OF UNSATURATED FATTY ACIDS IN MERISTEMATIC TISSUES.

In a previous paper (7) the abnormal curvature and swellings produced in roots and etiolated seedlings were dealt with and these phenomena associated with the incomplete formation of the primary endodermis usually present in these tissues, the unsaturated fatty acids instead of remaining in a strictly localised area in the endodermis diffusing outward and being found ultimately on other walls, even at the surface in the root sometimes. As a result the Casparian strip essential to the normal functioning of a primary endodermis, fails to form in some or all of the endodermal walls. Further experiment has shown us many cases of this effect but has also led us to realise that this failure of the Casparian strip must be considered as a secondary effect consequent upon the increased mobility of the unsaturated fatty acids within the walls of the meristematic tissue prior to the formation of the endodermis. As a result of the more rapid diffusion of these fatty substances from the meristem wall, this wall, especially in the etiolated stem, becomes distinctly more accessible to the aqueous sap by which nutritive material reaches the protoplast of the meristem. The result is seen in a more rapid growth of some of the meristematic cells and notably a more rapid vacuolation and extension in size as the result of the freer access of water to them. Two examples may be cited in which this effect has been clearly distinguished from the more secondary result that, owing to this migration of the fatty acids, the primary endodermis is not so complete as usual.

The etiolated seedlings of lentil (*Lens esculenta* Moench) were under observation as Coupin (1) had recently described them as taking up a horizontal position in relation to gravity. Under examination no trace of such an effect was obtained, the stems growing perfectly erect as those of other etiolated seedlings. History therefore seemed to be repeating itself and Coupin's observations might be due to a trace of coal gas or other impurity in the air such as earlier led Neljubow (5, 6) and the other German workers to the first thorough examination of the impurities in laboratory air which affected their experiments upon tropisms. Etiolated lentils were therefore grown in the presence of traces of coal gas and the shoots rapidly showed the marked horizontal direction of growth described by Coupin. Furthermore, swellings were visible upon the narrow etiolated shoots and these upon anatomical investigation proved to be developed one or two inches above the level at which the endodermis had as yet differentiated. In the case of the broad bean or pea the swollen region in a young seedling usually corresponds with the region where an endodermal break can already be found, but if older seedlings are experimented with it is also possible occasionally to find the swelling developing at levels higher than that at which the Casparian strip can be detected.

The second line of evidence as to the importance of the primary effect upon the mobility of fatty acids on the walls, as distinct from the effect in preventing endodermis formation, is supplied by a study of the effect of traces of coal gas upon etiolated seedlings of *Phaseolus multiflorus* Willd. as no primary endodermis is present in the etiolated epicotyl of this plant (Priestley and Ewing (9)).

Here again, however, traces of coal gas or of ethylene produce a marked shortening of the growth of the epicotyl together with a swelling of the shoot, though this may not be so markedly localised as in stems where it is associated with a structural break in the endodermis. The effect of traces of coal gas upon the etiolated shoots and roots of growing plants must therefore be traced primarily to the effect of the gaseous unsaturated hydrocarbons present, upon the mobility of the unsaturated acids in the developing walls of meristematic and differentiating tissues, one effect frequently associated with this being the partial or complete disappearance of the Casparian strip of the primary endodermis. The fact that stems growing in the light show no such effect would seem to mean that one effect of the light is to cause a rapid mobilisation of these unsaturated acids so that any subsequent further effect by the addition of traces of unsaturated hydrocarbons is rendered impossible. This effect of the light is in accordance with the difficulty in diffusion through the tissues of the apical meristem in the etiolated shoot which experiment has shown to exist, as compared with the ready diffusion along the walls of the green shoot in light (Priestley and Ewing(9), *loc. cit.* p. 32).

CORK FORMATION FROM A MERISTEM.

Before describing experiments upon the effect of coal gas upon cork formation, it will facilitate an explanation of these results if a brief account is first given of the developmental sequence of events in cork formation in a stem (Priestley and Woffenden(11)). The experiments subsequently described have all been carried out upon *Sambucus nigra* L., though many other plants have been occasionally used with concordant results. Events in cork formation can be similarly described for this one plant and thus precision given to the details. In general the essential points are common to all cases of normal cork formation in the stem.

In the young green twig the epidermis is covered with a cuticle, the only openings in which are the stomata; these occur all over the surface but are more numerous just below the node. Quite early in development, within two or three internodes of the apex, the substomatal air cavity shows an accumulation of fatty substance all over its floor and side, so that evaporation from the walls of the tissues bordering this air cavity must be considerably diminished in amount. It appears to be associated with this fact that a cork phellogen arises below a stoma long before it appears anywhere else upon the surface of the stem(11).

This phellogen cuts off regular tissue to the outside which gradually raises the floor of the substomatal cavity until the stoma is replaced by a lenticel, which at first appears as a pale coloured pustule on the surface of the internode, rapidly browning in colour as the cells outside the cork tissue dry up. The cork phellogen thus active below the stoma commences its activity in the centre of the substomatal cavity, spreading relatively slowly to the margin of the base of the lenticel; no further cork phellogen appears for some time, so that in the early summer, all the internodes from near the top to the very base of the current year's shoot are covered with lenticels in various stages of development. Later in the year, towards the end of July in Leeds in 1922, a cork phellogen arises throughout the surface of the stem, just below the epidermis, and so forms a continuous sheet of phellogen between the lenticels.

This new phellogen forms as a continuous cylinder, from the base upwards. Its passage up the stem can be traced by the colour change at the surface of the stem from green to silver grey. This change occurs following the first formation of cork cells from the phellogen, when air spaces appear between the epidermis and this new tissue beneath and modify the refraction and reflection of light from the cortical tissue.

No adequate explanation is known to us for this rapid formation of cork phellogen from the base of the shoot upward at this season of the year and the subject certainly seems to require investigation.

Whenever a cork phellogen becomes active, whether at the base of a lenticel or beneath the epidermis, the subsequent events seem to follow the same course. The cells of the phellogen are of the usual meristematic type, densely packed with protoplasm, with prominent nucleus and free from vacuoles (Priestley and Pearsall (10)).

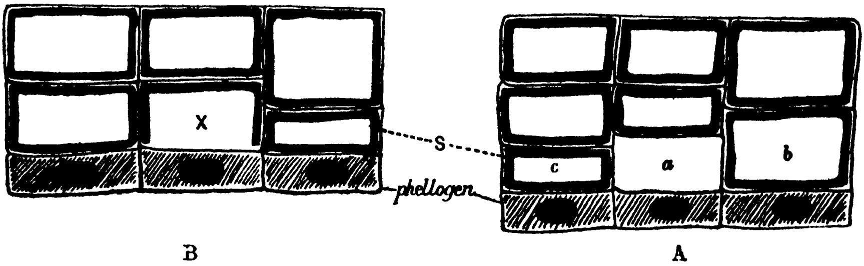

Fig. 1. Diagrammatic representation of cork cells cut off from phellogen. For description see text.

The wall gives no reaction for cellulose with chloriodide of zinc until after previous boiling with strong potash or prolonged treatment with Eau de Javelle. In this respect it behaves like other meristem walls (Tupper-Carey and Priestley (13)) and provisionally may be assumed to be a complex containing at least protein, cellulose, fatty acid and pectin. As the cells are cut off to the outside by this meristem they rapidly differentiate, the wall separates into two lamellae, an outer (middle) lamella, containing pectin and fatty acid, and an inner layer with a cellulose basis. At the same time large quantities of fatty acid are being liberated within the protoplast, containing, to judge from their reaction with osmic acid, considerable quantities of unsaturated linkages. These diffuse to the surface of the protoplast and there diffuse into and impregnate the two lamellae of the wall. As a result of the impregnation of the middle lamellae the cork tissue becomes very resistant to the normal macerating agents (acids followed by weak alkali or ammonium oxalate) which depend upon their power of forming free pectic and fatty acids and then removing these acids as soluble salts. The unsaturated acid impregnating the lamella oxidises and condenses to a form which resists the action of strong acids (unless oxidising agents are present) and is also stable to weak alkalis. Most of this acid is however deposited in and upon the original cellulose lamella, and as it there undergoes oxidation and condensation forms the characteristic suberin lamella (Priestley (8)). A succession of cells are thus cut off to the outside by the phellogen and undergo this process of differentiation, the contents being ultimately either deposited on the wall as the suberin lamella (*S* of text-fig. 1) and its cellulose basis and inner lining or left in the cell as granular débris of no further importance.

As a result of the fact that the suberin lamella is formed from fatty substances released at the surface of the protoplast, the lamella forms simultaneously around the cell. Thus close examination will show that a cell such as *a* in text-fig. 1 A has not yet commenced to deposit its suberin lamella and the first superficial appearance of three-sided suberisation is due to its position amongst cells (as *a*, *b* and *c* in text-fig.) already suberised. Close examination will show that such a cell as *X* in text-fig. 1 B with a suberin lamella on three walls, never occurs during cork formation. It is now possible to discuss the effect upon this process of cork formation in the elder stem of the presence of coal gas with its gaseous unsaturated hydrocarbons.

EXPERIMENTS WITH COAL GAS.

The experiments were carried out upon elder plants growing in the open, the gas supply being led out from a neighbouring laboratory (Leeds coal gas contains about 2 per cent. ethylene) and then divided into six streams by T joints, such streams passing through a washbottle and so to a tube fixed upon an elder stem. The gas supply was regulated by screw clips upon the rubber junctions and so arranged that a slow supply was delivered constantly during the period of each experiment. No attempt was made to work with any definite concentration of coal gas around the stems. The tubing around the gassed stem was about one inch in diameter, the leaves being cut down, except the youngest ones, to admit its insertion; the tube was closed with a split cork at either end, the gas being led in through a tube in the lower cork and allowed to escape freely through a hole in the upper cork. No special precautions were taken to make the tubes air-tight; control tubes of the same diameter were always placed at similar positions on other stems of the same plant and air was allowed to circulate through these. The experiments were all carried out in the period from July 1st to August 25th, 1922, the stems being examined after from five to nine days' confinement within the tube. When the younger internodes alone were under treatment, no leaves were cut off and the terminal shoot was just packed with cotton wool as it emerged from the top of the tube. When it was desired to compare the effect of gas upon the lower internode without removing the leaves, the shoots were enclosed within balloons of oiled silk, closed by corks at either end. These experiments require no further special mention until leaf fall is discussed, as the effects of coal gas upon cork formation were unaffected by the removal of the leaves.

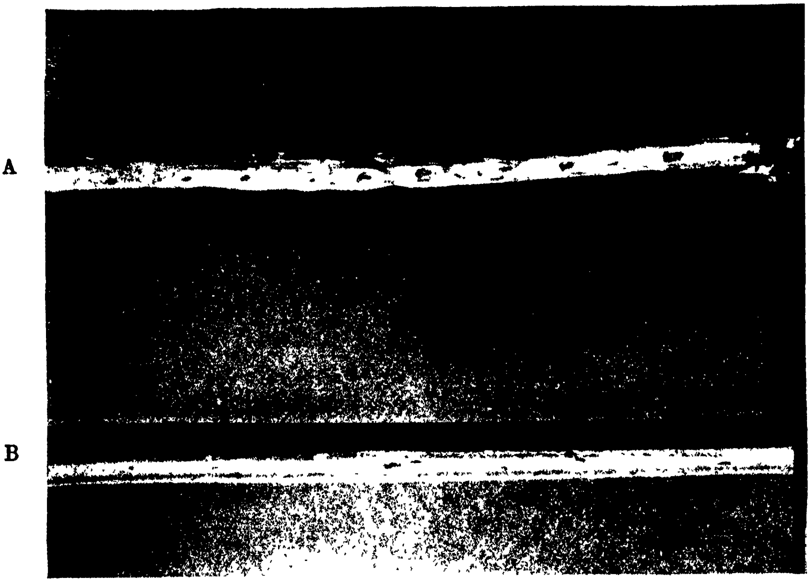

Fig. 2. Photo of elder twigs. A, exposed to coal gas; B, normal.

The result of the treatment with coal gas varied with the age of the internodes; three distinct types can be distinguished.

(i) Youngest internodes. When the apex of the shoot was exposed to the gas it began to droop within a few hours, later turning brown and withering. The stem immediately below the gassed nodes was nipped and brown and the stomata upon the gassed internode seemed to be sunk in little brown patches.

(ii) In tubes enclosing the internodes just below the apex and in any internode as far down as the fifth node before July 24th (1922), the effects of gassing were noticeable at the base of the petiole and the lenticels. The leaves had been removed, leaving from ¼ to ½ inch of petiole; almost invariably these dropped off within a few days, leaving a white spongy tissue covering the leaf-scar. The effect upon the lenticels varied with their age. The younger lenticels showed an eruption of white proliferated tissue in their centre, surrounded by a brown margin, the older ones showed a brown centre pushed up upon a white rim of proliferating tissue.

(iii) In the case of. all stems gassed after July 24th no effect was produced upon the lenticels of the lower internodes but gradually the stem surface between the lenticels became swollen and discoloured (text-fig. 2); this appearance always showed first at the base of the gassed region, spreading up the stem, and frequently travelling three inches within 24 hours. After July 24th the effect was always obtained until towards the end of August, then the colour of the stem showed that cork formation had already occurred close up to the youngest internodes and the experiments were stopped as the coal gas was now without effect on any but these youngest internodes.

The control stems enclosed within tubes, showed that the striking effects produced in these experiments could not be ascribed to the moister atmosphere within the tube; in these stems the only effect produced was an occasional indication of proliferated tissue on the lower side of the base of the cut petiole after the tubes had enclosed the stems for two or three weeks. Only in one case did this proliferation cause abscission of the remaining part of the petiole and then after 23 days in the tube whilst the coal gas experiments were all examined after a much shorter period.

The connection of the effect with the age of the internode suggests that the effect is only produced at certain stages of development. In the youngest part of the twig the effect seems to be mainly produced in the region of the stomata. Sections showed the brown discolouration to be due to the presence of dead disorganised cells, the nipped regions below the node seem to be due to the great loss of turgidity in this region where the stomata are most numerous. This particular effect has not been investigated further at present, the main object of the present investigation being the effect of the gas upon cork formation.

The effects upon the lenticel show the first example of the connection of gas poisoning with cork formation and show clearly that the effect is produced at a certain stage of the phellogen's activity. In the young lenticels the phellogen in the centre of the substomatal cavity is in the right stage and a mass of proliferated tissue appears in the centre of the lenticel. In an older stage, the cork in the centre of the lenticel is too far advanced to be affected and is simply carried up as a brown plug upon the proliferating cells resulting from the effect of the gas upon the activity of the phellogen near the margin of the lenticel.

Sections show that the proliferating cells are the cells cut off to the outside of the phellogen, which are practically completely unsuberised although on staining with Sudan III irregular accumulations of fat may be found within them.

Similarly when the lenticels are too old to be affected and the stem surface between is swollen, sections show that the epidermis in this swollen region is forced up by the large, proliferated, unsuberised cells, formed to the outside by the activity of the sub-epidermal cork phellogen. It is obvious that the normal differentiation of these cells is greatly modified when it has to take place in the presence of coal gas and that the main factor is again the inability of the unsaturated fats to deposit in the wall in the presence of the unsaturated hydrocarbons in the coal gas. This is further supported by the observation that whilst osmic acid shows very little unsaturated fat in these cells, if a strip of the epidermis be removed and the osmic acid applied to the inner surface, an instantaneous and very deep black reduction occurs, whilst if the epidermis be taken from the normal stem and a similar test applied, no reduction of the osmic acid is noted.

Evidently the unsaturated substances as they accumulate at the protoplast surface in the differentiating cork cells are no longer held upon the wall in the presence of the coal gas and so accumulate on the inner surface of the epidermal layer.

If the gassed and swollen stems are allowed to remain in normal air for some time, subsequent examination with Sudan III will show that the proliferated cells have gradually suberised although they are difficult to stain, the lamella being very thin and irregular.

It is apparently only those cells which are in a certain stage of differentiation when exposed to the coal gas in which distension takes place without suberisation, and if the phellogen has been active for some time before being exposed to the coal gas, outside the proliferated cells may be found not only unextended suberised cells but younger cells not yet suberised which have not undergone extension.

DISCUSSION.

Earlier work upon the effect of coal gas upon plants does not always provide sufficient anatomical data for exact comparison, but there can be little doubt that where these observers record effects upon the bark of woody plants, their results admit of explanation in the light of the experiments described above, and in every case are due to the cells arising from the cork phellogen originally proliferating and failing to suberise.

Doubt(2), studying the effects produced by coal gas upon the root system of young trees, notes that the underground portion of the stems frequently become soft and swollen, longitudinal splits appearing in the bark; the effects were very marked with apple, pear, ash and *Hibiscus*, less striking with *Sambucus*, *Grevillea* and cottonwood. In *Sambucus* and *Hibiscus* she reports the development of soft spongy tissues upon the lenticels.

Harvey and Rose(4) also noted that in woody plants the increase in diameter of the roots and the bases of the stems was mainly due to the rapid cell division of the phellogen and the elongation of the cells thus derived from this layer. They also noted proliferation at the lenticels upon the basal regions of the stems growing in the ground and thus exposed to the gas.

This proliferation has also been noted by Stone(12) when growing willow shoots in water contaminated with coal gas; as a result, in some cases there was a readier root formation from these shoots than from those in ordinary water, presumably because more water was absorbed through the proliferated and less suberised tissue of the lenticels.

Evidently these effects are all of the same type and in a growing root or stem system, which is clothed with a cork layer at a certain stage of development, some portion of the plant's surface will be susceptible to the toxic action of the impurities in the coal gas so long as growth is still proceeding. It is extremely unlikely that the contamination of the atmosphere with coal gas will ever be sufficient to make this effect of practical importance in the case of stem growing in air, even under greenhouse conditions, but toxic effects may undoubtedly result from leakages of coal gas around a root system, if the normal protection of this root system and of the basal portion of the stem is a cork layer. In this case some of the cork layer forming during the period of leakage will probably show these effects of proliferation and lack of suberisation and such a surface tissue would seem to invite the entry of pathogenic organisms.

Finally it may be pointed out that the abnormal tissues forming at the surface of plants in coal gas contaminated atmosphere, when the cork phellogen is active, is undoubtedly very suggestive in reference to the special tissue, described by Schenck as aerenchyma, which normally clothes some parts of the stem and root of marsh plants when they grow in stagnant water. Such water is frequently contaminated with hydrocarbons, some of them unsaturated.

SUMMARY.

1. Further experiments upon the effect upon the plant of atmospheres contaminated with coal gas, lend support to the view that this effect is mainly produced as a result of the mobility of unsaturated fatty substances in the plant walls in the presence of unsaturated gaseous hydrocarbons present in the coal gas.

2. Unsaturated fatty acids appear to be normal constituents of the meristem wall and in the presence of these unsaturated gaseous hydrocarbons they migrate towards the plant surface leaving the meristem walls more permeable. As a result, premature vacuolation and extension of the cells of the meristem take place. A failure of the primary endodermis in root and etiolated stem, owing to the absence of the fat impregnated Casparian strip, is frequently associated with this greater mobility of the unsaturated fatty acids. The normal green stem is indifferent to traces of coal gas because the fatty substances leave the meristem walls and accumulate in the surface wall at a very early stage of development.

3. The development of the cork phellogen in the stem of *Sambucus* is briefly traced, and the effect of coal gas upon this stem at different developmental stages is described.

4. The effects described are best interpreted on the assumption that at a very early stage of their formation, the cork cells cut off from the phellogen are very susceptible to the presence of coal gas. At this stage these cells become distended with water, and the fatty acids diffusing from the protoplast, fail to remain in and on the walls so that no suberin lamella is formed.

5. These results are entirely in accordance with the effects of coal gas upon the differentiating cells of the apical meristem of etiolated stem or root and can be explained by the greater mobility of unsaturated fatty acids in the presence of the gaseous unsaturated hydrocarbons present in the coal gas.

6. The experimental results are also in accord with those of earlier investigators. They suggest that coal gas will not be toxic to cork enveloped tissues except in the case of leakage from buried gas mains around the root systems of the plant; entry of pathogenic organisms will then be facilitated by the proliferated, unsuberised tissue formed from the cork phellogen.

7. The proliferated tissue formed from the cork phellogen in these experiments with coal gas appears to be suggestive in connection with the aerenchyma formed upon plant surfaces immersed in stagnant water.

REFERENCES.

(1) COUPIN, HENRI (1921). Sur une tige à géotropisme horizontal. *Comptes Rendus Acad. Sci.* CLXXII, 608–10.

(2) DOUBT, SARAH L. (1917). Response of Plants to Illuminating Gas. *Bot. Gaz.* LXIII, 209–24.

(3) HANSTEEN CRANNER, B. (1922). Zur Biochemie und Physiologie der Grenzschichten lebende Pflanzenzellen. *Meldinger fra Noyes Landbrukshiskole,* II, 1–160.

(4) HARVEY, E. M. and ROSE, R. CATLIN (1915). The Effects of Illuminating Gas on Root Systems. *Bot. Gaz.* LX, 27–44.

(5) NELJUBOW, D. (1911). Geotropismus in der Laboratoriumsluft. *Ber. der deutsch. Bot. Ges.* XXIX, 97–112.

(6) —— (1901). Ueber die horizontale Nutation der Stengel von Pisum sativum und einiger anderen Pflanzen. *Beih. zum Bot. Centr.* X, 128–38.

(7) PRIESTLEY, J. H. (1922). The Toxic Action of Traces of Coal Gas upon Plants. *Ann. of App. Biol.* IX, 146–55.

(8) —— (1921). Suberin and Cutin. *New Phytologist,* XX, 17–29.

(9) —— and EWING (1923). Etiolation. *New Phytologist,* XXII, 30–43.

(10) —— and PEARSALL, W. H. (1923). Meristematic Tissues and Protein Iso-Electric Points. *New Phytologist,* XXII, 185–91.

(11) —— and WOFFENDEN, LETTICE M. (1922). Causal Factors in Cork Formation. *New Phytologist,* XXI, 252–68.

(12) STONE, G. E. (1913). Effect of Illuminating Gas on Vegetation. *Report of the Botanist 25th Annual Report Mass. Agric. Expt. Station,* 13–28.

(13) TUPPER-CAREY, R. M. and PRIESTLEY, J. H. (1923). The Composition of the Cell-Wall at the Apical Meristem of Stem and Root. *Proceedings of the Royal Soc.* B, XCV, 109–31.

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FURTHER OBSERVATIONS ON THE PREVALENCE AND HABITS OF *OSCINELLA FRIT*, LINN.

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(With 3 Charts.)

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INTRODUCTORY REMARKS.

Many facts relating to the bionomics of the frit fly have been accumulated in recent years. The larvae live on cereals and grasses during the summer and on either grasses, volunteer cereal plants or late sown cereals during the winter. The flies emerge in maximum numbers in spring, midsummer and late summer but the relation of one generation to another in the field has not been accurately determined. The nature of the damage caused by the larvae is notorious, as also is the fact that early sown cereals suffer less infestation (from the practical point of view) than those sown late; but as yet we have to confess to ignorance of the factors controlling the difference in effective infestation in the two cases. We are equally in the dark as to the relative values of the many varieties of oat from the standpoint of susceptibility to infestation and power of recovery and the effect of these factors on subsequent yield.

The problems which present themselves for immediate solution are therefore (1) whether age of shoot is correlated with any morphological or biochemical variations which may be factors limiting infestation and (2) to what degree varietal differences influence infestation and yield under similar environmental conditions. Before knowledge gained by the solution of these problems can be applied in practice it is necessary to determine the variation in the field population throughout the year, the range of the maximum prevalence periods of the imago in the field from year to year and the relationship between maximum prevalence and maximum oviposition periods. Wild host plants, if heavily infested, may have considerable influence on the position of these maxima.

Data relating to the prevalence of the frit fly in the field have now been collected during the four consecutive years 1919 to 1922, which have shown wide variations in meteorological character. The observations made during 1919 and 1920 have been published (3) but are included in Chart I for comparison with the subsequent data. The interpretation of the first set of observations was difficult owing to the fact that the field data were apparently at variance with the experimental data and the more recent work has made it necessary to correct the views expressed in the previous publication (3).

Certain observations on the action of the larva on the host plant are also included.

A. THE PREVALENCE OF THE IMAGO IN THE FIELD.

Method of Procedure. The observations were again made on the University Farm, Sandford-on-Thames, the method of procedure being identical with that of the previous years (3). It is admittedly a crude method of determining population, but it is difficult to devise a more efficient yet practical procedure. In 1921 flies were collected on area G (3, fig. 1), but as this field did not carry spring oats in 1922, it was then necessary to sweep in a field on the same farm about ¼ mile north-east of area G.

1. *Data obtained by Sweeping.*

In 1921 Scotch Victory oats were sown on Feb. 2, succeeding roots in 1920 and winter oats in 1919, while in 1922 the same variety, sown on March 17, followed crops of roots in 1920 and 1921. In 1921 the oats matured in the middle of July, while in 1922 ripening was very slow, sweeping being continued until the end of August. The crops covered several acres in each case.

The smoothed curves in Chart I represent the prevalence of frit fly during the four years 1919 to 1922. Each point on the curves indicates the mean, over a period of seven days[1], of the number of flies caught while making fifty sweeps with a net twelve inches in diameter, on one or more occasions during that period. These seven day periods are reckoned arbitrarily from the first day of operation in 1919, and between the same dates in each year. In this way 22, 55, 20 and 3 per cent. of the points on the curves represent the mean values obtained from four, three, two and one field collections respectively, during the four years. The abrupt cessation of the curves is due either to the removal of the crop for economic reasons, or to the fact that when the seed is mature it is impossible to collect flies by this method owing to the rapidity with which the oats choke the net. Minimum grass, mean air and sun maximum temperatures and also rainfall, are shown in Chart II[1]. The method of smoothing necessarily differed from that adopted for the field data, as it was essential to include data for each day between the last days of collection in consecutive periods.

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[1] Seven day periods were taken in order to eliminate as far as possible variations due to unfavourable collecting conditions.

2 (*a*). *Interpretation of the Prevalence Curve for* 1922.

The form of a prevalence curve derived from field data obtained from a cereal crop may be dependent on several factors such as (1) the number of generations in the cycle, (2) meteorological variations, (3) variation in susceptibility of host plant, (4) influenee of wild host plants and (5) longevity of imago in the field. Meteorological variations, by altering the rate of development of the immature stages may cause variation in the periods occupied by the different generations and variations in the form of the curve. Variation in susceptibility of host plant may cause differences in extent of attack and movement of the period of maximum infestation. Wild host plants, to some extent, control the spring migration and by competition may affect the extent of oat infestation throughout the cycle. Longevity of the imago in the field will influence the form of the curve since maximum emergence and maximum prevalence periods will be separated by the longevity. If longevity is short, then emergence and prevalence curves will be nearly coincident.

As only the 1922 prevalence curve indicates the changes in population throughout a whole season it will be advantageous to consider this curve first and then compare the previous curves with it. In the first place there were no pronounced variations in the meteorological conditions during July and August to account for the maxima which were observed during these months and it is evident from our present knowledge of the bionomics of the fly that in this year the cycle was divided into three generations, distinguishable in the field about the periods ending June 13 and August 8. During the first period (May) Generation III appeared on the oats after hibernation during the larval stage in grasses. This was an immigration curve and may or may not have been (1) coincident with the emergence curve and (2) independent of the growth of the crop. A prolonged spell of high mean air temperature in spring evidently favours either fly production or collection, a short spell of high temperature in the spring of 1920 failing to produce a similar result[1], but observations made while the crop is young, necessitating sweeping close to the soil, are of doubtful value in indicating population. During the second period (June–July) the flies of Generation I emerged, the immature stages having fed on the young stems of oat and grass and the majority of these flies oviposited on the oat seed. During the third period (August) the flies of Generation II emerged, their larvae having been bred in the seed, late tillers or grasses. The prevalence curves of the years 1919 and 1922, during this third period, show very rapid changes of frit population on the oat crop. The curve here may be intermediate between the true emergence and the true prevalence curves and its form will be dependent on the relative attractiveness of cereal and grass. So late in the season, however, the oat crop will be unsuitable for oviposition and it is most probable therefore that the curve in this region approximates to the emergence curve, as emigration will be equivalent to short longevity in the field. The form of the curve at this season supports this view very strongly, there being very great disparity between the average number of flies present in the field during the periods ending Aug. 15 and Aug. 22, both in 1919 and 1922. Even if all the flies present during August 2–8 belonged to Generation II, the assumption that maximum emergence took place before maximum prevalence would imply that all the readings during August 9–15 were incorrect. As indicated in a previous paper(3) the development period decreases from 50 days in spring to 35 days in summer, which to some extent may explain the surge of Generation II in August. After August 29 the curve should no doubt fall much less rapidly, since flies may be taken in the field up to the middle of October (3, p. 108) but the collection of flies from oats becomes increasingly difficult as the seed matures. The very late individuals are probably the progeny of late members of Generation I, or perhaps early members of Generation II, in each case having fed on grasses in the larval state. It is obvious that the generations overlap considerably in the field.

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[1] Meteorological data supplied by the Radcliffe Observatory, Oxford, by the courtesy of the late Dr Rambaut.

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[1] The prevalence of the fly on *Lolium italicum* and also on barley in 1922 is recorded on Chart I. These data were collected on the next farm by the courtesy of Mr A. Kerwood and show (1) that the curves are not crop curves and (2) that immigration is rapid.

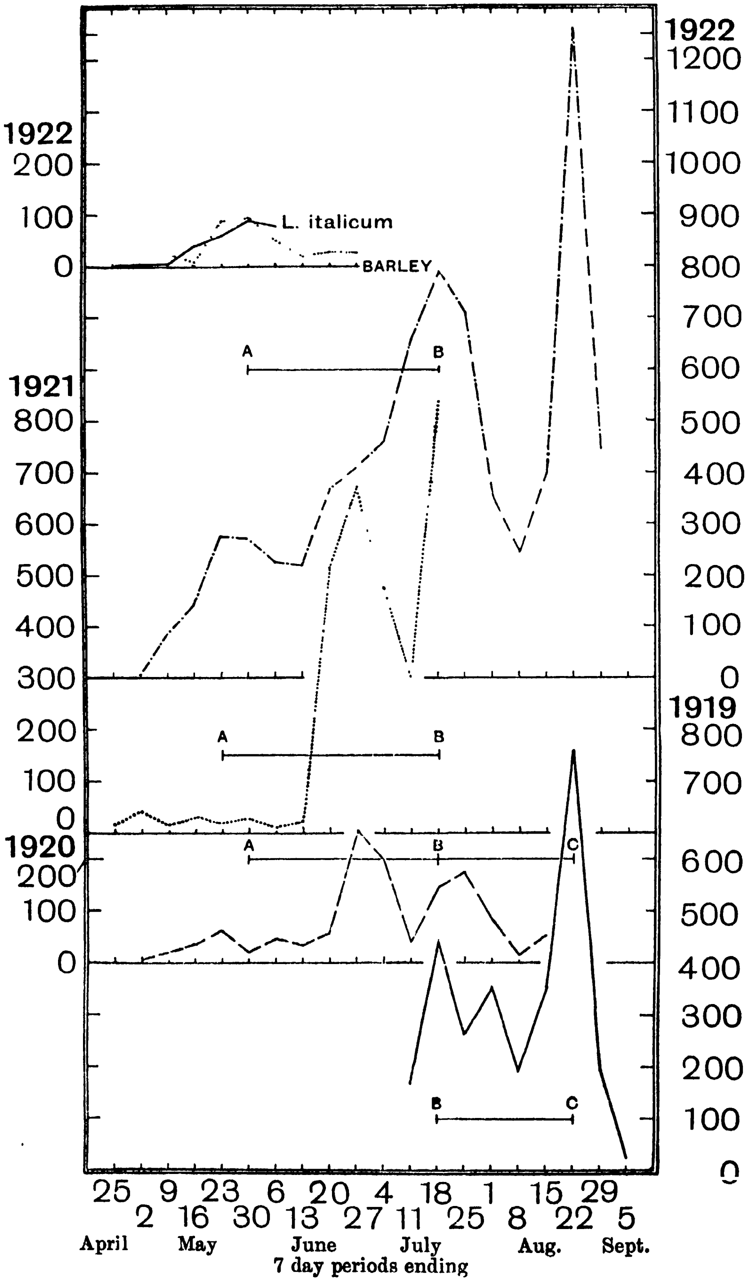

Chart I. Oscinella frit, Linn. Curves showing prevalence of imago on oats in the field, 1919–1922, and also on *L. italicum* and barley in spring 1922. A–B and B–C = average intervals in days required for the production of Generations I and II (determined experimentally) respectively.

Dates of sowing, 1919, May 2: 1920, Mar. 10: 1921, Feb. 2: 1922, Mar. 17.

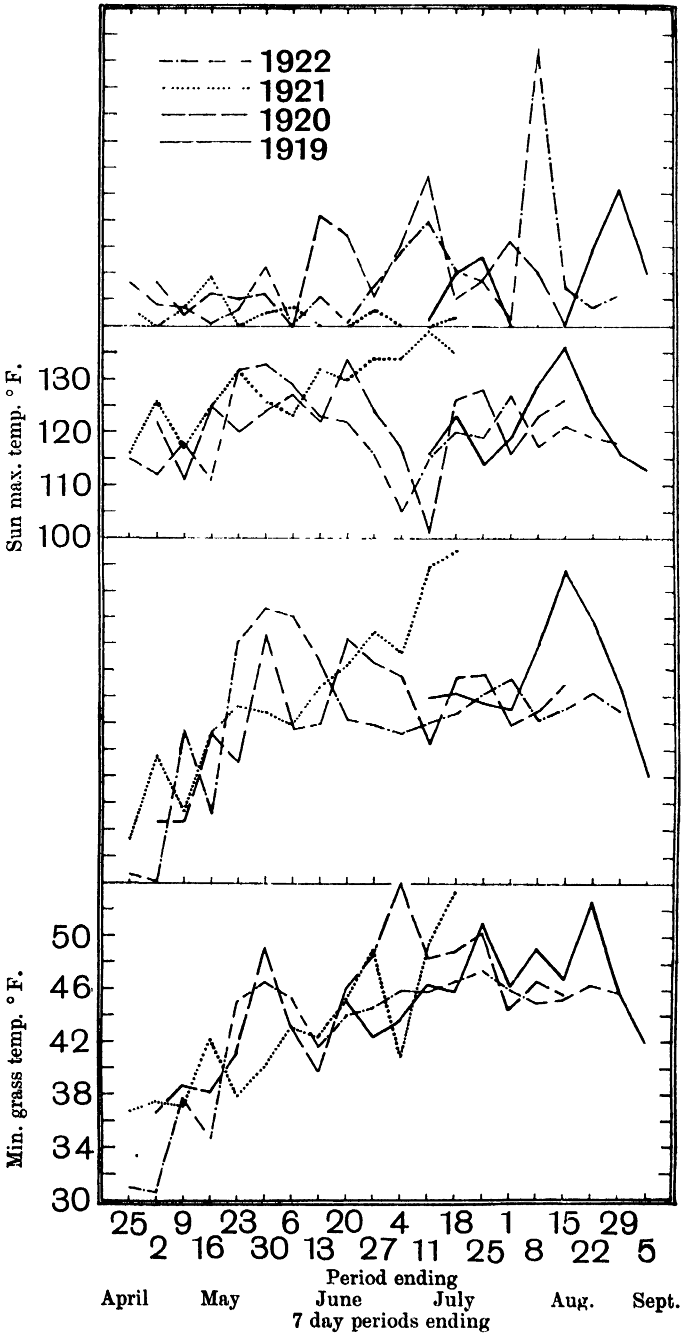

Chart II. Meteorological data, 1919–1922.

We may now consider experimental evidence collected during the four years 1919 to 1922 in relation to this interpretation of the curve. The results of outdoor breeding experiments are shown on Chart I under the different years, the periods A–B and B–C representing the mean number of days between the emergence of the consecutive generations, I from III and II from I respectively. In each case the parent flies were taken direct from the field in order that the oviposition periods in the cages and in the field might be comparable. To simplify comparison the points B are all placed in the period July 12–18, A and C being reckoned from July 15[1]. From these data it is clear that oviposition by Generation III at the end of May should result eventually in the appearance of Generation II in the third week in August and that this is strictly in accordance with the prevalence curve for 1922, in which year the prevalence maxima occurred about May 26, July 15 and August 19, *i.e.* at intervals of about 50 and 35 days respectively[2], these periods being in agreement with those obtained experimentally. Since the prevalence curve for Generation II appears to approximate closely to the emergence curve, the inference to be drawn from these data is that the periods about July 15 and May 26 were the periods of maximum emergence also for Generations I and III respectively, field longevity being short. The actual position of the period of maximum oviposition is undeterminable from these data, but it is known that the fly commences oviposition three to five days after emergence, thus the periods of maximum emergence and oviposition are probably closely approximate.

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[1] Synopsis of experimental data, abstracted from previous publications [3, 4 and 5].

1919.	Grasses infected	23. vii. 19.	B–C = 35·5 days	(51 flies).
1920.	Cereals infected	15. v. 20.	A–B = 50 ,,	(11 ,,).
	Oat panicles infected	14. vii. 20.	B–C = 33·4 ,,	(3 ,,).
1921.	Grasses infected	18. v. 21.	A–B = 58 ,,	(55 ,,).
1922.	Oats infected	16. v. 22.	A–B = 47·7 ,,	(22 ,,).

In the case of grasses sown April 20, 1922, and examined June 11, 1922, only 34·5 per cent. of the larvae had pupated by the latter date [5].

[2] The dates on which maximum collections were made in the field were, in order of the years 1919 to 1922; in May, —, 22, 13 and 24 equal, 23: in July, 17, 19, 18 and 12: in Aug. 20, —, —, and 21. In years when conditions were good for collection, these maximum figures were far above the next highest figures, as shown in the following table, where the lower of the two highest readings in the period is expressed as a percentage of the maximum.

	May to June 13	June 14 to Aug. 8	Aug. 9 onwards
1919	—	42·5	47·7
1920	94·9	79·8	—
1921	74·6	47·6	—
1922	51·7	68·4	74·0

On examining the temperatures[1] during the periods A–B and B–C it was found that excepting minimum grass temperature they were all lower in the second period than in the first, although the second period was 15 days shorter than the first. It would appear therefore that temperature is not the only controlling factor, but that the length of the maturation period may be dependent also on either qualitative or quantitative variations in the food supplied by the host plant or even on periodicity in the cycle. These are problems amenable to experiment which await solution. As indicated in Section B, the larva almost invariably attacks at the point where sap is likely to be most abundant. Baumberger (1) has demonstrated that in the case of *Drosophila* larval longevity is determined by the nitrogen supply and the fact that in this case the fly transfers its activities from stem to seed suggests that the availability of nitrogen may be the limiting factor when temperature is more or less a constant[2].

2(*b*). *Prevalence Curves during* 1919 *to* 1921.

It now remains to ascertain whether the above interpretation will apply also to the changes observed in the field population during each of the years 1919 to 1921. By comparison of the curves shown in Chart I it will be seen that the minima of the periods ending June 13 and August 8 are in close agreement, that in all years except 1920 the July population reached a maximum during the period ending July 18[3], and that in 1919 and 1922 the numbers of Generation II reached a maximum during the period ending August 22. But in the period ending July 11, the curve for 1922 shows no minimum, those for 1921 and 1920 pronounced minima, while in 1919 the curve may or may not be normal. Thus the question naturally arises as to whether a minimum should occur normally during the period ending July 11.

The data obtained from the outdoor breeding experiments (Chart I) would lead one to expect much the same grouping of the different generations in each year, and particularly in the years 1920 and 1922. The larva is an internal feeder and its rate of growth will be determined

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[1] Mean temperatures in 1922 in ° F.:

	May 23 to July 15 (A–B)	July 15 to Aug. 19 (B–C)
Min. grass	45·6	46·0
Min. shade	49·3	48·3
Mean air	59·2	56·0
Max. shade	67·2	65·8
Max. sun	122·0	120·0

[2] The grass experiment in 1919 (p. 60, note 1) does not negative this suggestion, as the temperature conditions were uncertain.

[3] In 1921, field observations ceased during this period owing to the removal of the crop. Also see footnote (2) p. 60.

mainly by the factors, availability of suitable food and temperature. Food in the form of either cereal or grass is always abundant, and as each type of host plant may be utilised by each of the generations(3) imaginal swarming at any rate within the limits of the third and first generations would appear to be determined mainly by temperature. Temperature will affect (*a*) the rate of maturation in winter and spring and consequently the period of maximum immigration of Generation III and (*b*) the rate of development of Generation I and thereby the position of the maximum emergence period of this generation.

Year 1919. In 1919 and 1922, the corresponding parts of the curves are almost identical. In 1919 sweeping was commenced during the period July 5–11 but although the mean population was small during this period it does not necessarily follow that Generation I was only commencing emergence at this date. The oats sown on May 2 would have been above ground about fifteen days on May 26 and certainly liable to infestation, maximum or otherwise. There seems to be no reason for assuming that Generation III failed to attack the crop at this date or that infestation was due mainly to late members of Generation III plus early members of Generation I taking advantage of maximum susceptibility. In the latter case both maxima should have appeared later in the year, as the mean air temperature for the period A–B was intermediate between the temperatures for the same periods in 1920 and 1922[1]. For the period B–C it was 6° F. higher than in 1922, but the increase was due to high temperatures towards the end of the period only (from August 8) when its effect would be comparatively slight, influencing the pupal stage only. In addition, in view of the 1920 and 1922 observations and the fact that the mean air temperatures were closely comparable, the period occupied by Generation I in 1919 was much too short for swarming to have commenced during July 5–11. As the maxima of August 16–22 and July 12–18 agree in 1919 and 1922, it follows that the spring maximum prevalence in 1919 occurred about May 26 again.

Year 1920. The mean air temperatures for the periods A–B and B–C for the two years 1920 and 1922 vary only by 2° F. the period B–C being cooler in 1922 than in 1920 and the period A–B *vice versâ*, while

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[1] Mean air temperature in ° F.:

	Prev. Sept. to April	May 23 to July 15 A–B	July 15 to Aug. 19 B–C
1919	43·5	58·4	62·2
1920	45·0	57·4	58·0
1921	46·3	61·6	68·7
1922	45·2	59·2	56·0

the crop conditions were very similar. There is then no reason to assume from temperature considerations that the minimum of July 5–11 in 1920 marks the end of a generation; it is due to the fact that the point is dependent on one field collection only, made when weather conditions were very adverse, the weather generally about these dates being cold, wet and windy. The facts that the period July 3–8 was recorded (3, p. 109) as being "too wet for sweeping" and that the weather was rough when sweepings were made in the adjacent periods seem to be very probable explanations of the whole of this depression. This being the case, the field and experimental observations taken in conjunction indicate that in 1920, as in 1922, the maximum prevalence of Generation III occurred about May 26. The slight increase in Generation I shown during the period ending June 20 is dependent on two divergent readings only and may actually have been more than is indicated in Chart I.

Year 1921. The records obtained in this year are of particular interest owing to the very exceptional meteorological conditions prevailing during the growing period, but they are however somewhat difficult to interpret. This year was characterised by an abnormal drought and by exceptional heat in July which resulted in the early sown oat crop ripening by the middle of July.

There are two possible interpretations of this curve, namely that (1) the minimum observed in July 5-11 may have been real in the cycle, *i.e.* the maxima may have represented the prevalence maxima of separate generations I and II or (2) the record of July 12–18 may have been, or may have been approaching, the maximum prevalence of Generation I only. In view of the exceptional season one would perhaps naturally conclude that the first theory is the more probable, particularly as mature flies were emerging in small numbers from the ears by the middle of July[1]. But the evidence is not in favour of this theory, although on the other hand there is some difficulty in determining definitely the cause of the July minimum from the facts available.

If the first theory interprets the curve correctly consideration of the experimental evidence in conjunction with the temperature data makes it necessary to assume that the maximum spring immigration occurred at the latest in the period ending May 9. If immigration were normal then the rate of development of Generation I must have been accelerated greatly.

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[1] **The first record of flies emerging from ears collected in the field was July 9. On July 18, 1921, the field was sampled in the quarter sections and in the centre, twenty panicles being collected at random in each position. After being rubbed out, 100 seeds of each sample were examined—infestation 19, 17, 18, 19 and 16 per cent. (centre). On Aug. 25, 1922, two samples showed infested seed to the extent of 58·7 and 52·8 per cent. respectively.**

Early Immigration. The mean air temperatures for the period September to April, during which Generation III is immature[1], for the years 1919 to 1922 were 43·5°, 45·0°, 46·3° and 45·2° F. respectively. These figures are in agreement with the early commencement of immigration in 1921, but it will be noted that the advancement is slight. The period of maximum spring prevalence will depend also on the temperature[2] prevailing during the pupal period, pupation occurring during March and April. For these months the mean air temperatures for 1920 to 1922, were 46·6°, 46·9° and 41·5° F. respectively[3].

Thus pupal development should have been much the same in 1920 and 1921 and delayed in 1922, thereby causing the postponement of the period of maximum prevalence in the latter year. But actually the 1920 and 1922 results are closely comparable, the temperature variations being therefore insufficient to affect the grouping of the generations. Thus the position that maximum immigration occurred about May 9 appears untenable, as otherwise one would have expected (*a*) to find flies in the field about the second week in April and (*b*) to have observed the surge of Generation I about May 17–23.

Clark, Adames and Margary (2) have shown as the result of phenological observations in 1921, that a season causing earliness in botanical phenomena does not necessarily cause correlated variation in insect cycles. The meadow-brown butterfly, the first appearance of which is recorded as normally occurring on June 12 in the Midlands, in this year appeared one day later (*in. litt.*). The longevity of the larva, which is the hibernating stage, was therefore normal in 1921.

Finally one may bring forward corroborative evidence in the shape of records[4] of the prevalence in 1921 and 1922 of an allied species with very similar habits, namely *Chlorops taeniopus* Mg., the larva of which mines in the stems of barley and couch grass (Chart III). In 1921 the fly appeared on the crop as soon as it showed above ground (May 20) and the spring generation disappeared on June 17 in 1921 and on June 20 in 1922, showing that there was in this case only slight advancement of fly development in the spring of 1921. The summer generations of flies appeared first on July 24 in 1921 and on July 30 in 1922, closely approximate dates considering the smallness of the infestation and the meteorological variations. Thus the maturation periods of this fly also were comparatively unaffected by the abnormal season of 1921.

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[1] The longevity of the hibernating larval stage doubtless enables the different individuals to negative age differences to a great extent. In 1921 the mean air temperature for the development period of Generation II, July 15 to August 19 was 7·5° F. above the mean for the four years, but emergence was not correspondingly earlier in the spring of 1922.

[2] A study of the "effective temperatures" was confirmatory but afforded no additional information on either of these points.

[3] Mean minimum grass temperatures, March–April, 1920, 36°: 1921, 35°: 1922, 31° F.

[4] Data collected on a neighbouring farm by the courtesy of the owner, Mr A. Kerwood. In 1921 the barley was sown on May 10 and in 1922 on April 5.

The balance of evidence appears to be against the probability of undetected early immigration.

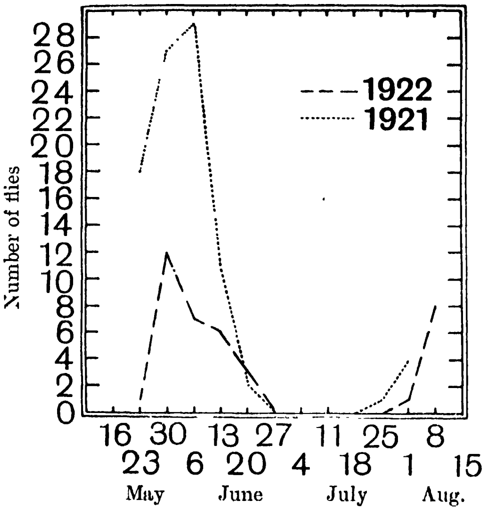

Chart III. Chlorops taeniopus, Mg. Curves (smoothed as in the case of *O. frit*) showing prevalence of imago on barley in the field, 1921 and 1922.

Accelerated Development. The alternative explanation that the apparent early swarming of Generation I was due to an increased rate of development caused by high temperature is excluded by the fact that the mean air temperature[1] for the period concerned, April 26 to June 27, in each of the years 1920 to 1922 was 55·8°, 56·6° and 56·9° F. respectively, the temperature differences being negligible compared with the 21 days difference in the appearance of the maxima in 1921 and 1922. The high mean air temperatures (above 70° F.) of this year occurred after July 8 only, too late to be effective in shortening the period for Generation I. In

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[1] Maximum shade temperatures for the same periods, 1920 to 1922, 63·1°, 66·7° and 66·0° F. It should be noted that the surge of Generation I is comparable in all years. For the period May 23 to June 27, mean air temperatures 1920–1922 were 58·3, 58·6 and 60·6° F. respectively.

1921 the minimum of July 5–11 was apparently real in the field as the population decreased from June 28, seven collections being made between June 28 and July 11, a period favourable for collection. It is difficult to determine the cause of this decrease. It cannot be accounted for on the basis of non-activity, as the sun maximum temperatures were abnormally high during this time (see Chart II), while in 1922 very low temperatures in the period ending July 4 failed to produce a minimum. Minimum grass temperature however was very low (40·8° F.) for the period ending July 4, 1921, and it was only at the end of the succeeding period that recovery took place. This might check both pupal development and emergence and would explain the subsequent minimal prevalence, field longevity being short[1]. On the other hand, in May 1922, immigration increased rapidly from the 2nd onwards at a very low minimum temperature. The mere fact that the flies were present in large numbers when the grain was ripe would appear to be evidence in favour of the first theory. But if the majority of these flies belonged to Generation II, it follows that they must have been the progeny of Generation I swarming in maximum numbers during the period ending June 27. The improbability of Generation I having reached a maximum here has been indicated above. The fact that the fly was breeding in the seed in July does not necessarily imply that the majority of the flies collected in the middle of July belonged to Generation II. The oats were in flower at the beginning of June and these flowers would be sought as oviposition sites by late members of Generation III, the progeny of such individuals having sufficient time to mature by mid-July[2]. The grain infestation in 1921 was light compared with 1922 (footnote, p. 63), while the population was much the same in each year. If immigration were normal, then the July maximum was normal also, the flies being members of Generation I bred in late tillers. At the same time, additional immigration from wild host plants may have occurred.

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[1] The correlation coefficient between prevalence over the period June 14 to July 18 and minimum grass temperature advanced one period is $+0{\cdot}68 \pm 0{\cdot}16$. Although the p.e. is high owing to the small number of mean values the high positive coefficient, while not very significant, would appear to support this view. In 1922 this minimum was absent and in May there was a single immigration maximum only. These facts are in agreement with the experimental evidence in both years and indicate that the minimum is not explicable on the assumption that the fly has double broods normally [3, p. 111]. Also as the spring immigration extends over a long period and as the fly is always present in the field except in winter, the curve should ascend regularly throughout the summer if more than one brood is normal.

[2] A minimum period of 41 days was observed during experimental breeding between the times of emergence of consecutive generations at this time of year [3, p. 110].

An interpretation based on variation in susceptibility to infestation, with consequent early infestation in 1921 seems to be precluded by the fact that the prevalence in August 1919 was comparable with that in August 1922.

The interpretation of the 1921 curve is admittedly a little uncertain, but from the data available it would appear that it represents the prevalence of Generation III and I and is therefore comparable with the other curves.

It may be concluded that the 1922 form of curve represents the normal summer prevalence undisturbed by adverse meteorological conditions.

3. *Constancy and Duration of Prevalence Periods.*

Viewed broadly these curves show a similitude which is remarkable considering the meteorological characters of the different years. The effects of these meteorological variations on the plants were more marked than in the case of the prevalence periods of the fly. The swarming of a new generation is definitely marked and strikingly constant in the different years, which means that the meteorological variations of the years 1919 to 1922 were not sufficiently diverse to move these points of the cycle out of the seven day periods.

Thus the field evidence shows that between May and August the frit fly has only three generations. In the imaginal stage Generation III extends from the period April 19–25 to the period June 7–13, with a maximum prevalence about May 26. Generation I extends from the period May 31–June 6 to the period August 9–15 with a maximum about July 15. Generation II extends from the period July 26–Aug. 1 to the period Sept. 6–12 with a maximum about Aug. 19. There may be a partial fourth generation between Generation II and Generation III (which hibernates in grass host plants) being the progeny of the early individuals of Generation II.

4. *The Relation of the Fly to its Host Plants.*

The immigration in spring of Generation III from wild grass hosts to the oat crop is independent of the state of the crop in regard to growth, and the period of maximum infestation seems to be more or less constant in time. This is important in relation to the yield of the plant as the main stems and early tillers may thereby escape attack in the case of early sown crops. The later tillers will of course always be liable to infection to a degree dependent on the time of their appearance in relation to the period of maximum oviposition. Thus the state of the crop can only influence the extent of attack (from the point of view of yield) through variation in susceptibility. If increase in age causes a diminution in susceptibility (5), one would expect an early sown crop to produce a greater number of stems capable of shooting than a late sown crop. In the former case attack would be confined chiefly to tillers which would in any case have a low yield value, whereas in the latter case the main stems and first tillers would bear the brunt of the attack, while the extent of attack might be the same in each case. That the fly desires to oviposit on the oat rather than on suitable wild grasses[1] has been shown in a previous note (5) not to be true invariably. It appears more probable that the fly roves in search of oviposition quarters and that it will readily accept grasses in lieu of oats, if the former are more suitable for its purpose. If the form of the prevalence curve were dependent on susceptibility of host plant alone, one would have expected in 1922, when the oats were sown on March 17, that the emergence of Generation I would have been earlier than in the case of oats sown late as in 1919 (May 2). The fact that this is contrary to the field data obtained in these two years does not necessarily mean that variation in susceptibility of stem does not exist but that it may be obscured by the availability of a succession of tillers or grass stems suitable for breeding. In the case of the grain infestation a similar explanation will not suffice, since if the grain (which is formed in a short period) is beyond the stage desired for oviposition when the maximum prevalence occurs in July, the swarm of flies in August (Generation II) would be dependent on an earlier infestation by a smaller number of flies of Generation I. The maximum prevalence of Generation II should then be earlier in the case of an early flowering crop than in the case of a crop with a susceptible stage at or beyond the maximum prevalence period. Since the curves in 1919 and 1922 are identical at this period the inference is that prevalence in August is dependent on grasses as well as on oats.

Thus one is led to conclude that the fly is ready to utilise oat stems or oat grain if these are available at the right time, and if not, suitable wild or cultivated grasses; that the prevalence is therefore dependent on the total number of host plants present and not on oats alone; and that the nature of the curve is on the whole dependent more on wild than on cultivated host plants. While the growth of the oats in spring may or may not affect the extent of the attack on the stems yet it determines the yield, inasmuch as yield is dependent on the number of stems capable of shooting as well as on the extent of the attack on the grain. The attack on small or later tillers may maintain intensity without causing substantial loss in yield. If the crop could be protected from the fly in spring, the latter would find abundant food supplies in its wild host plants and swarming again in June and July, would be ready to attack the grain. Trap crops are therefore unlikely to be of much value in combating this pest, because of the ease with which the fly can adapt itself to any one of its numerous wild host plants and the enormous reservoir of flies which must be maintained in such plants.

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[1] Grasses in which the fly has been bred experimentally are recorded in (3) and (4).

B. THE ACTION OF THE LARVAE ON THE HOST PLANT.

Stem. In the field in spring, the majority of the eggs are found on the lower blades, living or dead, the number of eggs recorded per blade varying from one to thirteen, although numbers above three are rare. Later in the season eggs may be found on the upper blades, sometimes on the sheaths and even attached to the haulm near or just below the soil surface. When the panicles appear the eggs are laid inside the glumes. The attachment to the plant is very definite and doubtless aids the larva to free itself from the eggshell. On emergence the larva immediately seeks the cover of the sheath and penetrates to the region of the growing point, where the supply of sap is plentiful.

All types of entrance galleries have been observed. When the egg is deposited on the haulm near the ground, the entrance hole is visible and the gallery may be traced through the successive layers until the growing point is reached. More commonly, after gaining entrance, the larva cuts through the tissues in a horizontal spiral to the centre of the stem, thereby cutting off supplies from the youngest blades. In about 12–15 days external evidence of this attack is apparent owing to the consequent discoloration. In the former case there may be no external signs of attack or only slight flagging of the central leaf and this may lead to errors as large as 30 per cent. in estimating extent of attack[1], as the fly is present in the field from April to October. At the other extreme, the larva appears to work its way down from the blade between the sheaths and commence its attack well above the actual growing region. Whether it eventually includes the growing region seems to depend on the vigour of the shoot. This type of damage is usually confined to the older stems, and may be considered abnormal[1]. In these cases the appearance of discoloration is of course dependent on the site chosen by the larva; panicle damage cannot be detected at this stage. The rapidly growing larva extends the gallery in the central shoot, the region of working almost invariably including the youngest tissues. If the stem is vigorous, the visible central shoot may suffer lateral damage only, in which case subsequent discoloration would be slight.

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[1] On June 8, 1920, thirty plants were taken at random in the field. One or more larvae were found in 23 shoots but 29 per cent. of the latter showed no external sign of damage. In every case the larva had destroyed the growing region but the central leaf was killed only in those cases in which the larva had excavated a gallery round the base [see also (3) p. 122].

The range of shoots capable of utilisation by the larvae is very wide. Small tillers one-third of an inch in length and still enclosed in the sheaths have been found to contain larvae, even when the main stem has been free from attack. Presumably there is no question of preference here, the young tiller happening to be in the line of attack. Usually when the attacked stem is young, its growth is severely checked if not entirely inhibited and the side tillers are forced into growth abnormally. These in turn may be attacked before emergence from the sheaths if the principal stem does not afford the larva sufficient nutriment. Of the larger stems, namely those twelve inches or more in height, as many as one-fifth may be damaged beyond recovery[1]. When the attack is the result of oviposition by late members of Generation III or early members of Generation I, the developing panicles enclosed in the sheath are partially or wholly destroyed as shown by Roebuck (6).

The degree of destruction of the central leaf, the typical external sign of attack, depends partly on the method of entry and subsequent working of the larva and partly on the state of development of the plant.

Grain. It was noted when larvae were reared in captivity on fresh oat grains, that for the first three to five days they preferred to work along the groove and between the pericarp and the endosperm. In the few cases under observation, they subsequently extended their working into the endosperm in the region of the embryo. It is in this region that the conducting vessels enter the seed. In field samples this preliminary boring is most noticeable when the grain is either approaching or in the milk stage. When the attack occurs after fertilisation, but before growth is pronounced, the grain continues to receive food supplies until the vessels at the base are destroyed; dwarfed grain results and the endosperm may be entirely consumed by the larva. If the ovule is attacked before or immediately after fertilisation, the larva destroys all parts of the flower which is consequently sterile[1].

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[1] During variety trials in 1923, the following data were obtained from oats sown on 18. iv. 23: number of stems examined = 2064; number of stems showing signs of injury exclusive of growing point = 40 or 1·95 per cent.; number of stems 12″ and over in height including blades = 473 or 23 per cent.; number of latter damaged = 102 or 21·6 per cent.

Several seed samples were tested by measurement of their germinative capacity to ascertain whether the larval method of working in the grain invariably killed the embryo, different varieties, including awned varieties, being examined. From a sample of the variety "Abundance," with a germinative capacity of 72·6 per cent., a thousand grains were thrown into water to separate the sound from the damaged seed, which resulted in 55·1 per cent. sinking. The floating seeds were skimmed off and 150 seeds of each type were germinated under similar conditions. The germinative capacity of the heavy grain was 97·6 per cent., and of the light grain 41·8 per cent.[2] Examination of the seed which failed to germinate showed that of the heavy grain 2·4 per cent. of the seed had been slightly attacked, the embryonic region at the base alone being decayed, while of the floating grain 54 per cent. showed signs of attack in different degrees and 4·2 per cent. failed although apparently healthy. In a sample from "Excelsior" seed purchased for farm use (normal germinative capacity 93·2 per cent.), 15·5 per cent. of floating grain was obtained. The germinative capacities of the heavy and light sections were 97·4 per cent. and 69 per cent. respectively and in both cases the seed which failed showed decay at the base originated by frit attack. In neither case did any of the germinated seed show any sign of attack.

It would appear therefore that the larvae exhibit a pronounced tendency to attack those parts of the plant receiving an abundant supply of sap. The factor which determines the choice of site by the female, for the deposition of eggs, is at present unknown[3].

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[1] It has been proved experimentally that a larva can reach maturity in this manner. The panicle of an oat plant was caged immediately after flowering and infected with a pair of flies. The plant bore 16 flowers, from which were bred five flies. Examination after the emergence of the latter showed that 11 grains were untouched, averaging 1·2 cm. in length; while of the five damaged grains, four measured 0·7, 0·7, 0·6, and 0·4 cm. respectively and one flower showed no development, even the anthers being destroyed.

Specimens of this type are encountered fairly commonly in field material, the empty puparia being usually present.

[2] The radicles of the germinated "Abundance" seed gave the following minimum, mean and maximum measurements in the same period: (1) heavy 0·1, 0·69, 1·4; (2) floating, 0·1, 0·4, and 1·1 cm. The "Excelsior" radicles averaged (1) 0·99 cm. and (2) 0·71 cm. The percentages of floating grain by weight were for "Abundance" 51 per cent. and for "Excelsior" 10·6 per cent. Thus in the latter case, where the original germinative capacity was high, 10 per cent. of the seed by weight was of poor value as seed either through frit attack or inherent weakness and could be utilised more profitably for other purposes.

[3] Chemotropic experiments, using ether extracts from stems of known age, gave negative results in 1923.

SUMMARY.

1. Observations on the prevalence of the imago of *Oscinella frit* in the field have been made during the period 1919 to 1922.

2. The annual cycle consists of three generations: Generation III swarms from the end of April to the middle of June, with a maximum about May 26; Generation I from the end of May to the middle of August, with a maximum about July 15; Generation II from the end of July to the beginning of September (or longer) with a maximum about August 19.

3. Although meteorological variations during the period 1919 to 1922 were apparently very diverse, they were not sufficiently diverse to affect the prevalence periods of the fly on the crop materially; the prevalence curves of the different years show a remarkable similarity. This may be due to the fact that the larva is an internal feeder.

4. As the periods of maximum prevalence are not displaced from year to year, it would appear that the fly population is determined by the total number of host plants and not by the cultivated plants alone.

5. The larva exhibits a strong tendency to attack those regions of the plant which are in receipt of abundant supplies of sap.

REFERENCES.

(1) Baumberger, J. P. (1919). A Nutritional Study of Insects. *Journ. Expt. Zool.* xxviii, 1–81.

(2) Clark, J. E., Adames, H. B. and Margary, I. D. (1922). Report on the phenological observations in the British Islands, Dec. 1920 to Nov. 1921. *Quar. Jour. R. Meteorol. Soc.* xlviii, No. 204, 293–327.

(3) Cunliffe, N. (1921). Preliminary Observations on the Habits of *Oscinella frit*, Linn. *Ann. App. Biol.* viii, No. 2, 105–34.

(4) —— (1922). Additional Host Plants of *Oscinella frit*, Linn. among Grasses. *Ibid.* ix, No. 2, 165–8.

(5) —— (1923). On the Relative Importance of Certain Common Grasses as Host Plants of *Oscinella frit*, Linn. *Ibid.* x, No. 2, 210–12.

(6) Roebuck, A. (1921). Frit fly (*Oscinis frit*) in Relation to Blindness in Oats. *Ann. App. Biol.* vii, 178–82.

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THE INFLUENCE OF HYDROGEN-ION CONCENTRATION ON THE GROWTH OF CERTAIN BACTERIAL PLANT PARASITES AND SAPROPHYTES

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(With 3 Text-figures.)

In the course of a series of determinations of the points of maximum acid-agglutination for a number of species of bacteria, some of which were plant parasites, it was noticed that there seemed to be a distinct relationship between acid agglutinability and pathogenicity to plants. Although further work did not entirely confirm the view that tolerance of high hydrogen-ion concentrations is always a distinguishing characteristic of bacterial plant parasites, yet the important part which hydrogen-ion concentration probably plays in the defence of the plant cell against invading bacteria, seemed to make it of interest to find out how far it affects the growth and activity of different types of such bacteria.

It seemed possible that the acid limits of growth, and the hydrogen-ion concentrations producing agglutination would fall within the range of cell sap acidities in the case of non-parasitic species and above it in the case of pathogenic varieties. The following determinations were therefore carried out with 28 species, fourteen of which were parasites, and the others saprophytic organisms of common occurrence.

I. The determination of the points of maximum acid agglutination.
II. The determination of the acid limits of growth.
III. Determinations of the changes in hydrogen-ion concentration accompanying growth.

I. DETERMINATION OF THE MAXIMUM POINTS OF ACID AGGLUTINATION.

This series of tests had been originally begun in the hope that agglutination with acid would prove a ready means of identifying different species of bacteria. The points of maximum agglutination were not, however, found to be specific, but, as stated above, there seemed to be a marked relationship in many cases between agglutinability and pathogenicity to plants. The series of determinations was therefore completed for 28 species representative of the following classes of organisms:

1. Fourteen plant parasites, most of which had been isolated in the laboratory. The cultures of *B. marginale* and *B. viridilividum* had been given by Miss N. Brown (6); the type here called "*Barkeri*" was a sub-culture of the organism causing pear blossom blight, isolated by Prof. Barker (2) while the *Aplanobacter Michiganense* strain was obtained from Dr Erwin Smith (19).
2. Four non-parasitic members of the *fluorescens* group, viz., *B. fl. liquefaciens*, *B. fl. non-liquefaciens* (*putidum*), *B. pyocyaneus* and *B. violaceus* (*janthinus*).
3. Five common sporing soil organisms.
4. *B. coli*, *B. prodigiosus*, *B. lactis aerogenes*, *B. faecalis alcaligenes*.

The fourteen species in the last three sections were all obtained from the National Collection of the Lister Institute.

The method used was that recommended by Michaelis (15) for the identification of typhoid and paratyphoid bacteria; but as his acetate solutions give only a short range of *p*H values, from 3·5–5·0, and the mixed series used by Bondorff (5) which included acetate, lactate, tartrate, and citrate mixtures, do not, as he admits, give concordant results, Sørensen's buffer mixtures of HCl and acid sodium citrate giving a range from *p*H 1·0 to *p*H 5·0 were employed. The mixtures of the two reagents were made up in the proportions 10 : 0, 9 : 1....0 : 10, giving *p*H values as indicated in Dr Walpole's chart of hydrogen-ion concentration data (22). All the strains of bacteria tested gave agglutination optima within the range of these citrate solutions except *B. mesentericus* which agglutinated in a mixture of acid sodium citrate and sodium hydroxide at *p*H = 5·4, and in a mixture of sodium acetate and HCl at 5·6.

The emulsions used were made from bouillon agar cultures of about 18 hours' growth; the growth was not washed off, but removed with a platinum loop, so that change of *p*H value due to admixture of water of condensation was avoided. Each of the agglutinating tubes received one part acid solution and three parts emulsion, these were incubated at 30° C. for four hours, or less, if agglutination was found to be beginning, and then allowed to stand at room temperature overnight. In some cases, when agglutination was very slow, the tubes were raised to a temperature of 37° C.

The following table gives the *p*H values at which maximum agglutination occurs in the citrate solutions in the case of the 30 species tested:

	Parasitic species	Non-parasitic species
*p*H = 1·2	Ps. phaseoli B. Barkeri B. atrosepticus	B. pyocyaneus
*p*H = 1·5	Aplanobacter michiganense B. solaniolens	B. prodigiosus
*p*H = 2·0	B. Tolaasi B. aroideae B. campestre B. marginale B. viridilividum B. solanisaprus B. caratovorus	B. fl. liquefaciens B. fl. non-liquefaciens B. violaceus B. coli communis B. lactis aerogenes
*p*H = 3	B. lathyri Ps. proteamaculans	
*p*H = 3·7	Ps. hyacinthi	B. coli communior B. faecalis alcaligenes B. subtilis B. vulgare
*p*H = 4·8–5·0		B. megatherium B. dendroides B. mycoides
*p*H = 5·4		B. mesentericus

The maximum agglutination points are not sufficiently well defined to be characteristic of individual species and therefore, as Bondorff concluded, the method is of very limited use as a means of identifying different kinds of bacteria. The majority of plant parasites showed marked resistance to the influence of acid; agglutination visible with a hand lens (magn. 10 diam.) began in these cases at about *p*H 3·0 and reached its maximum at *p*H 1·5–2·0. Above this point, at *p*H 1·4, flocculation did not take place or was very slight, and in the case of nine parasitic species tested, death of the organism occurred in less than half an hour.

The agglutination of the four non-parasitic members of the fluorescens group was similar in all respects. The sporing organisms on the other hand, with the exception of *B. subtilis*, are agglutinated by much lower hydrogen-ion concentrations, lying between the values *p*H 4·8 and *p*H 5·6.

The agglutinating points of coli-form organisms, as given by several investigators, usually lie between these two extremes, viz.:

Typhoid	*p*H 4·40	Michaelis (15) Beniasch (3)
Paratyphoid and paracoli	3·50–3·80	Michaelis Bondorff (5)
B. coli (two strains)	2·96	Dreyer (9)
,,	2·6–3·0	Bondorff
B. faecalis alcaligenes	3·5	Beniasch

As will be shown later on there seems to be definite correlation between the resistance of an organism to acid agglutination and its power to produce acid or alkali from nitrogenous food material.

II. DETERMINATION OF THE LIMITS OF GROWTH DUE TO HYDROGEN-ION CONCENTRATION.

In these and the following series of tests, Jardox peptone broth containing ·5 per cent. meat extract and 1 per cent. peptone was mainly used as culture medium; 2 per cent. peptone water was at first used, but the soil organisms gave little growth in it.

No salt was added to the broth, as the concentrated Jardox already contains 8–10 per cent. and growth at the higher degrees of acidity was found to be checked by the addition of a further amount. The presence of larger quantities of salt in the medium may account for the lower acid limits of growth given by Wolf and Shunk in the case of *B. carotovorus* and *B. campestre* (23); possibly also for the very low values given in the British Drug Houses list for *B. coli*, *B. pyocyaneus*, *B. faecalis alcaligenes* and *B. vulgare*.

The temperature of incubation of the cultures was always 19°–21° C. as this approximates to the natural temperatures at which these bacteria live.

All the *p*H values were determined colorimetrically, using the indicators recommended by Clark (7). About ·5 c.c. of the culture medium to be tested was withdrawn with a sterile pipette and placed in a watch-glass, and to this a drop or two of the indicator solution was added, taking care that the culture liquid was distinctly in excess. The colour of the liquid in the watch-glass, placed on white paper could then be easily matched with the colours of Clark's chart, and differences of *p*H value to within ·1 could be estimated. Whenever possible two determinations were made with different indicators.

By using watch-glasses instead of test-tubes, difficulties in matching, due to turbidity, colour of the medium, reflections from the surface of the tubes, and dichroism of the indicator were largely avoided.

Series of standard tints in test-tubes made up according to Gillespie's (10) recommendations were used to check results especially when growth limits were being determined.

The points between *p*H = 4·0–4·8, which include the acid limits of growth for many species, are especially difficult to estimate owing to the dichroism of bromphenol blue; in these cases the culture tests were also matched with the colour given by the same quantities of citrate buffer solutions placed in watch-glasses.

The escape of ammonia or volatile acids is naturally more rapid from watch-glasses than from test-tubes and the readings should therefore be taken at once.

In cases where the cultures were tested from day to day the inoculations were made in duplicate or sometimes in triplicate, in order to guard against errors due to contamination when introducing the pipettes.

II a. *Acid Limit of Growth.*

The degree of acidity at which growth ceases was determined by inoculating tubes of broth acidified with HCl to *p*H values 4·0, 4·4, 4·6, and 5·0, with the species to be tested, and incubating them for at least a fortnight. At *p*H – 4·0 no growth was obtained in any case.

The following list gives the highest degree of acidity at which growth was obtained:

	Parasitic species	Non-parasitic species
*p*H = 4·4	B. solanisaprus	B. coli (5·0 BDH)*
	B. atrosepticus	B. fl. liquefaciens
	B. lathyri	B. fl. non-liquefaciens
	B. campestre	B. pyocyaneus (6·0 BDH)
*p*H = 4·6	B. Barkeri	B. prodigiosus
	B. caratovorus	B. dendroides
	B. Tolaasi	B. lactis aerogenes
	B. solaniolens	B faecalis alcaligenes (6·9 BDH)
	B. viridilividum	
	Ps. protea-maculans	
*p*H = 4·8	B. marginale	B. vulgare (6·5 BDH)
*p*H = 5·0	Apl. michiganense	B. violaceus
	Ps. phaseoli	B subtilis
	Ps. Hyacinthi	B. mycoides
		B. megatherium
		B. mesentericus

* BDH indicates the value given in the list published by the British Drug Houses, Ltd.

It is of interest to note that no growth was obtained in any case in broth of acidity greater than *p*H 4·4, the value given by Pearsall and Priestley as the iso-electric point of many of the principal proteins of plant tissues (16).

As in the previous series of experiments a greater tolerance of acid is shown by most of the plant parasites and the types allied to *B. fluorescens* than by *B. vulgare* and the sporing species; the parasitic forms *Ps. hyacinthi* and *Ps. phaseoli*, however, seem particularly sensitive to acids, a fact noted by Dr Erwin Smith (20).

Advantage may be taken of the fact that plant parasites are more resistant to an acid culture medium than the common sporing organisms, to free cultures of the former from such contaminations. If material from a diseased plant be inoculated into broth at pH 4·6–5·0 any growth during the first or second day will be practically free from such sporing organisms, as the latter only give a visible turbidity in about five days at $p\text{H} = 5{\cdot}0$.

This treatment before plating is useful in the case of the "soft rot" and *Pseudomonas* groups, but is naturally of no avail with slow growing forms like *Aplanobacter* or *B. viridilividum*.

II b. *Alkaline Limits of Growth.*

The degree of alkalinity at which growth in peptone broth ceases was also determined; it was found to be approximately the same, *i.e.* pH 9·3–9·5 for all the species tested, both parasitic and non-parasitic, with the following exceptions:

B. faecalis alcaligenes	pH = 9·6 (9·7 BDH)
B. vulgare	= 9·2 (8·8 BDH)
Ps. hyacinthi	= 9·2
Aplanobacter michiganense	= 9·2
B. mycoides	= 8·9
B. viridilividum	= 8·9
B. pyocyaneus	= 8·8 (9·0 BDH)
Ps. phaseoli	= 8·7

Difficulty was experienced in getting the medium free from the turbidity due to the addition of alkali, but by mixing $N/10$ NaOH with broth in the proportions 1 : 3; 2 : 2; and 3 : 1 and then autoclaving, three series of tubes containing broth at $p\text{H} = 8{\cdot}2$, 9·0, and 9·7 were obtained in which the turbidity was precipitated out and growth could be observed in the clear medium above. The hydrogen-ion concentration of these increased slowly, probably owing to absorption of CO_2 from the air; when the first trace of growth appeared, a small quantity of the broth was removed with a sterile pipette and its pH value tested colorimetrically. Every test was repeated and confirmed again in the course of later work.

In some cases free growth in the liquid medium was preceded by a slow growth on the surface of the glass, this was especially noticeable with *B. mycoides* and *Aplanobacter michiganense*. It was at first mistaken for a deposit from the broth. Sodium hydroxide appears to render the cell walls of the bacteria mucilaginous, but instead of agglutinating with one another they settle on the glass walls of the tubes. Attempts were made to get agglutination with alkaline solutions, but only a film of bacteria adhering to the glass was obtained in most cases, apart from chemical precipitates which were often very misleading. In spite of this action of caustic soda on the cell walls, the bacteria are not quickly killed; emulsions of eight representative species were still living after three hours' immersion in decinormal sodium hydroxide.

III. CHANGE OF HYDROGEN-ION CONCENTRATION DUE TO BACTERIAL GROWTH.

It had been noted in previous work(4) that bacteria which were agglutinable in Michaelis' acetate solutions slowly developed an alkaline reaction during growth in peptone water, while certain species which were inagglutinable within the range of these solutions (pH 3·50–5·00) gave no alkaline reaction when tested with α-naphthol phthalein even at the end of three weeks. Twenty species were therefore inoculated into tubes of peptone water, the original pH value being 6·9. Each day small quantities were tested with the reagent.

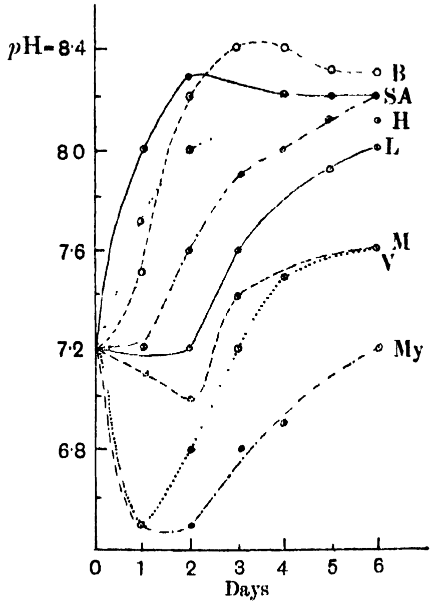

Fig. 1 a.

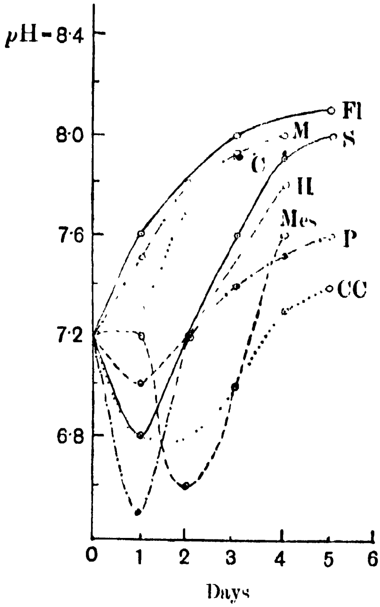

Fig. 1 b.

Fig. 1 a. Changes in pH value of cultures in peptone water during the first six days of growth. *B* = *B. Barkeri* (1·2); *S* = *B. solaniolens* (1·5); *A* = *B. atrosepticus* (1·2); *Fl.* = *B. fluorescens liquefaciens* (2·0); *L* = *B. lathyri* (3·0); *M* = *B. megatherium* (4·8); *V.* = *B. vulgare* (3·7); *My* = *B. mycoides* (5·0).

Fig. 1 b. Changes in pH value of cultures in peptonized Jardox broth during 4–5 days' growth. *Fl* = *B. fluorescens non-liquefaciens* (2·0); *M* = *B. marginale* (2·0); *C* = *B. carotovorus* (2·0); *S* = *B. subtilis* (3·7); *Mes* = *B. mesentericus* (5·4); *CC* = *B. coli communior* (3·7); *H* = *Ps. hyacinthi* (3·7); *P* = *B. prodigiosus* (1·5).

Eight plant parasites and *B. fl. non-liquefaciens* gave the blue colour indicating a fall of hydrogen-ion concentration below $pH = 7{\cdot}3$ by the second day, *B. lathyri* gave it on the third, *B. coli* on the fifth, while three "ammonifying" organisms *B. mycoides*, *B. subtilis* and *B. vulgare* only gave coloration after the seventh day.

Further experiments confirmed this result that the bacterial species with high agglutination maxima rapidly produced alkali in peptone water, while the "ammonifying" organisms with lower agglutination points produced acid during the first day or two of growth and only later gave rise to an alkaline reaction in the medium.

The curves in fig. 1 represent these changes in pH value, due to the growth of several selected species of bacteria, in 5 c.c. of Dunham's peptone water, and fig. 2 gives similar curves for bacteria growing in peptonized Jardox broth. In the latter medium better growth was obtained, particularly in the case of the sporing organisms, but the changes in pH value were less marked, and the final pH values for the plant parasites were lower. These final pH values are always considerably lower than the alkaline limits of growth, probably because other toxic substances accumulate in the medium.

The agglutination optima of the various species are given in brackets in figs. 1 and 2, so that the correspondence between these and the rapidity of alkali formation can be readily recognized. In this series *B. prodigiosus* is exceptional in its behaviour. It resembles the parasites in its high maximum agglutination point, but forms acid, like the sporing forms, during the first two days of growth. Later experiments showed that it reacts to acid solutions by the production of alkali, but in neutral and alkaline media such as here used it produces acid.

ACID AND ALKALI PRODUCTION IN NUTRIENT MEDIA OF VARYING HYDROGEN-ION CONCENTRATION.

In order to determine whether the initial acidity of the medium had any influence on the formation of alkali during growth, tubes of peptonized broth, brought to various pH values with HCl and NaOH, were inoculated with a loopful of a 24 hour broth culture of each species; these were incubated at 21° C. for two days and the change of pH then determined.

The reaction of the *Pseudomonas* and *fluorescens* group to acidity of the nutrient medium is very marked, as is shown by the curves in fig. 2, where the abscissae represent the initial pH values of the medium, and the ordinates the change of pH in the cultures at the end of two days' growth; positive ordinates represent alkali formation, negative ones the development of an acid reaction. It will be seen that the cultures giving greatest alkali formation are those of high initial acidity, not those of maximum growth, as evidenced by the earliest appearance of turbidity, and greatest turbidity at the end of 48 hours.

Rapid alkali formation in acid media is also a characteristic of the parasitic forms allied to *B. caratovorus*, but in this group there seems to be a slight tendency to form acid in alkaline media.

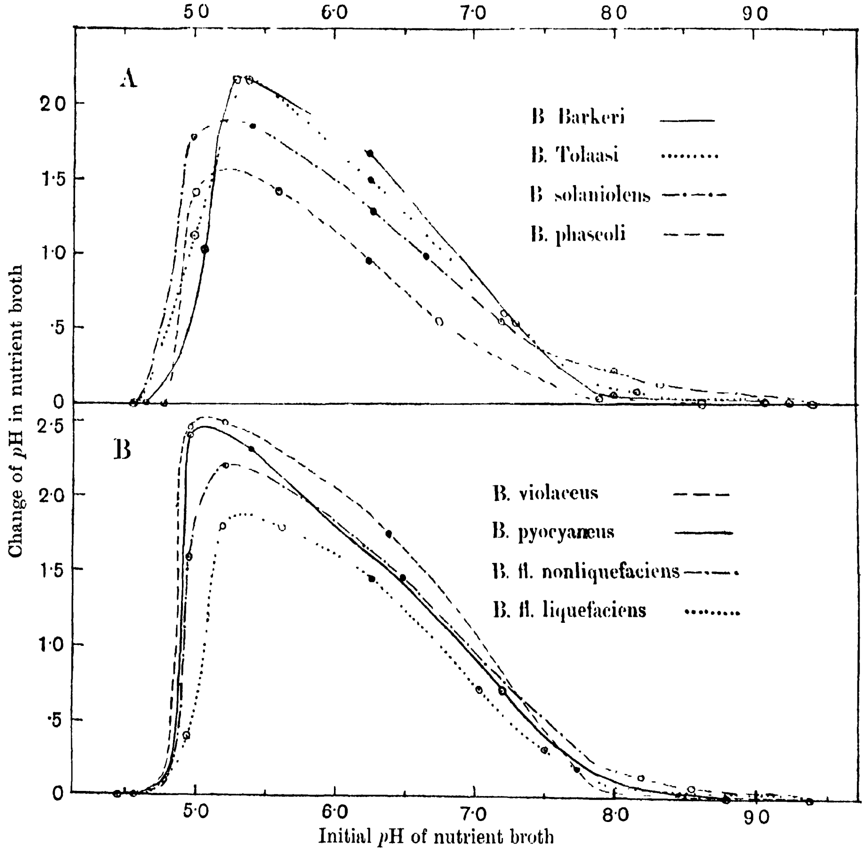

Fig. 2. Showing alkali production by various members of the *Pseudomonas* and *B. fluorescens* group in peptonized broth of varying hydrogen-ion concentration after 48 hours' growth. A. Plant parasites. B. Non-parasitic species. ● Cultures showing maximum growth.

Most of the sporing species and also *B. vulgare*, on the other hand, form acid at all hydrogen-ion concentrations during the first two days of growth; the curves in fig. 3 illustrate the contrast between the behaviour of these organisms and that of the plant parasites. *B. dendroides*

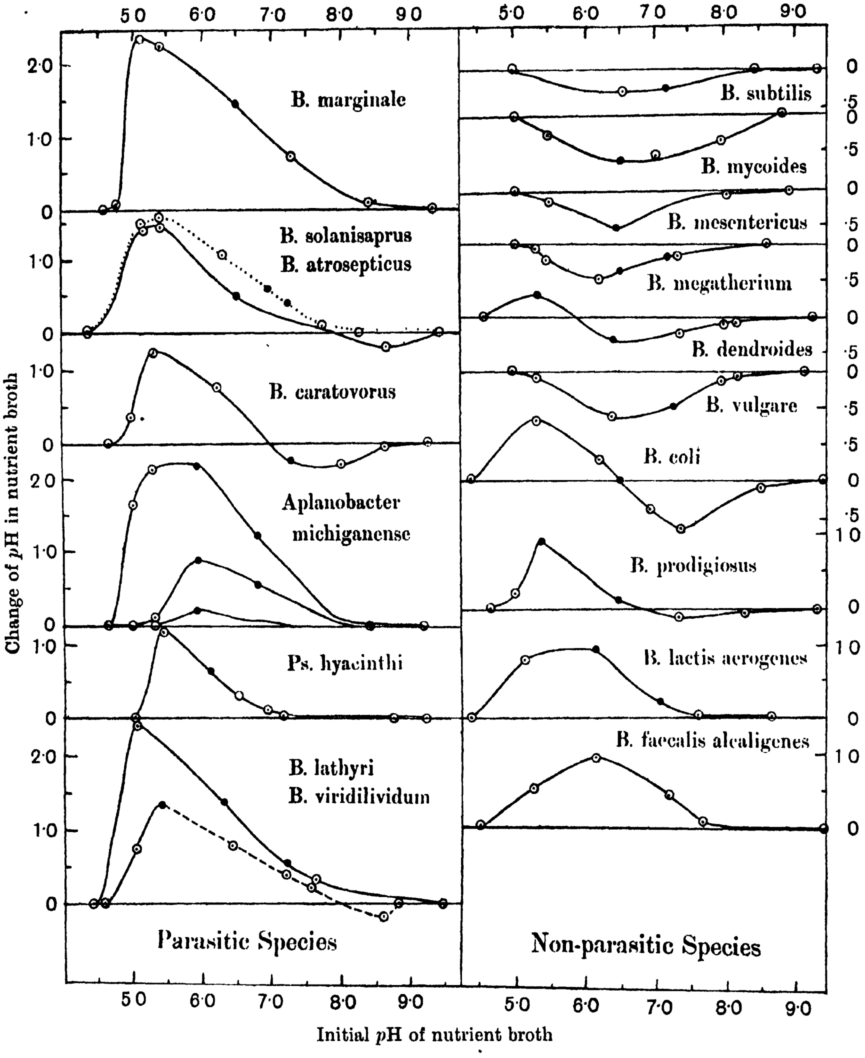

Fig. 3. Showing alkali or acid production by various species of bacteria after 48 hours' growth in peptonized broth of varying hydrogen-ion concentration. Positive ordinates = alkali production. Negative ordinates = acid production. ● Cultures showing maximum growth.

appears to be an exception among the sporing organisms, like *B. coli* and *B. prodigiosus* it forms alkali in acid media and acid when the media is initially alkaline.

The non-parasitic forms *B. lactis aerogenes*, and *B. faecalis alcaligenes* were found to form alkali just as freely as many plant parasites.

SUMMARY AND DISCUSSION.

It is interesting to see in the foregoing series of experiments that most of the bacterial plant parasites, especially those of the *Pseudomonas* type, and the non-parasitic species of the *fluorescens* type seem to possess three associated characteristics, namely high acid agglutination points and limits of growth, and a capacity to produce alkali rapidly in the presence of nitrogenous food materials. This latter capacity is shown in a less marked degree by other non-sporing saprophytic organisms such as *B. lactis aerogenes*.

Many sporing species, on the other hand, appear to possess a different physiological constitution, since they produce acid in the earlier stages of growth in sugar-free peptonized broth, and are characterized by comparatively low agglutination points and growth limits. Tests by Marchal and other observers (14) at a later period of their development have caused these sporing organisms to be regarded as the chief ammonia producers in the soil.

The experiments also show that the hydrogen-ion only begins to act injuriously on the bacteria at concentrations about *p*H 5·0–4·4. Such hydrogen-ion concentrations are seldom met with in plant tissues except in the case of acid fruits. The acidity of the cell sap has been determined by several investigators by the hydrogen electrode method; Clevenger (8) found that it varied from *p*H 4·33 for Buckwheat seedlings to *p*H 6·40 for Soy bean leaves. The values obtained by Hoagland (11) for barley tops, by Phillips (17) for Indian Corn and *Vicia faba*, and by Wagner (21) by means of an indicator method for *Sinapis alba*, *Brassica oleofera*, *Solanum tuberosum* and *Sempervivum Hausmanni* all lie between *p*H 4·9 and *p*H 6·2. The cell sap used in these experiments was generally obtained by pressure of the tissues after grinding with sand; treatment of sections of fresh tissues with methyl red indicates that the acidity of the protoplasm itself is somewhat higher than that of the cell sap, whilst Atkins (1) found that the liquid centrifuged from the tracheae of *Acer pseudoplatanus* was neutral to litmus. Priestley (18) too remarks with respect to the sap collected from a bleeding vine stem, that, "tested with the usual series of indicators, the *p*H values of the sap in the vines was practically that of true neutrality."

The higher acidities of these plant extracts would be a serious check to growth and multiplication in the case of the sporing species only; they are quite too low to cause the death or even the agglutination of most of the bacteria here tested.

The low hydrogen-ion concentration in the vessels of the wood may account for the fact that the bacteria causing wilt diseases are at first confined to the vascular bundles, where they can multiply freely, they only attack the more acid parenchymatous tissues later when the latter have become disorganized by the blocking of the water channels.

It has been shown, however, by certain investigators that reduction in the acidity of the cell sap does, in some way, facilitate the attack of bacteria on plant tissues. Laurent(12) found that *B. coli communis* and other non-parasitic bacteria would rot slices of raw potato which had been immersed in a 1 per cent. solution of Na_2CO_3 or K_2CO_3 for one hour, but as he found that there was no relation between the total acidity of the juice of different varieties of potato and their resistance to the attacks of *B. coli* and *B. fluorescens putidum*, he concluded that this resistance was due to the existence in the cell sap of a soluble substance whose power was annihilated by alkaline substances.

Lepoutre(13) confirmed Laurent's work, and also claimed to have shown that turnips and carrots grown on plots treated with lime or salt or nitrogenous manures were less resistant to bacterial invasion, and were even attacked by the species *B. fluorescens liquefaciens*, *B. mycoides* and *B. mesentericus*, usually regarded as non-parasitic. On the other hand he considered that treatment of the soil with phosphoric acid rendered the plants less susceptible to infection.

Therefore, although it is quite obvious that the power to produce alkali rapidly from nitrogenous food materials does not by itself enable bacteria to live parasitically in plant tissues, since both parasitic and non-parasitic species may possess it in the same degree, yet it may be an important factor in enabling bacteria invading an open wound to produce an environment favourable to their own growth and development before cork formation limits their activities. In the case of the sporing species and *B. vulgare* the tendency to form acid from nitrogenous food materials as well as from carbohydrates, coupled with their agglutinability in comparatively low hydrogen-ion concentrations, would seem to render them incapable of attacking living plant tissues.

The writer wishes to express her thanks to Dr S. G. Paine for the interest and criticism so kindly given in the course of the work.

LITERATURE.

(1) ATKINS, W. R. G. (1916). *Some recent Researches in Plant Physiology*, p. 188. Whittaker & Co., London.

(2) BARKER, R. I. P., and GROVE, O. (1914). A bacterial Disease of Fruit Blossom. *Ann. App. Biol.* I, 85.

(3) BENIASCH, M. (1912). Die Saure-Agglutination der Bakterien. *Zeitschr. Immunitäts*, I. Abt. (orig.), 12, p. 268.

(4) BERRIDGE, E. M. and GLYNN, E. E. (1920). Observations on the Classification of 48 strains of Flexner-Y and allied Bacilli. *Journ. Path. and Bact.* XXIII.

(5) BONDORFF, K. A. (1917). Om Syre-agglutinationens Anvendelse ved den Bakteriologiske Arts-diagnose. *Den Kgl. veterinaer og landbohjskole Aarskrifft, Saertryk.*

(6) BROWN, N. A. (1918). Some bacterial Diseases of Lettuce. *Journ. of Agric. Research*, XIII, No. 7, 367.

(7) CLARK, W. M. (1920). *The Determination of Hydrogen Ions.* Williams & Wilkins, Baltimore.

(8) CLEVENGER, C. B. (1919). The Hydrogen-Ion concentration of Plant Juices. *Soil Science*, 8, p. 217.

(9) DREYER, G. and JEXBLAKE, A. J. On the Agglutination of Bacteria. *Det Kgl. danske Vidensk. Selsk. Skrifte, Naturvidensk. og mathem.*, Afd. 7, R. I. 4. Quoted by Bondorff (5), *Journ. of Pathology and Bacteriology*, XI, 1.

(10) GILLESPIE, L. J. (1920). Colorimetric Determination of Hydrogen-Ion Concentration without Buffer Mixtures, with especial reference to Soils. *Soil Science*, 9, p. 115. Also *J. Am. Chem. Soc.* XLII, 742.

(11) HOAGLAND, D. R. (1919). The Relation of the Nutrient Solution to the Composition and Reaction of Cell sap of Barley. *Bot. Gaz.* LXVIII, 297.

(12) LAURENT, M. E. (1899). Recherches expérimentales sur les Maladies des Plantes. *Ann. de l'Inst. Pasteur*, t. XIII, 1.

(13) LEPOUTRE, B. (1902). Recherches sur la Transformation expérimentale de Bactéries banales en Races parasites des Plantes. *Ann. de l'Inst. Pasteur*, XVI, s. 304.

(14) LÖHNIS, F. (1910). *Handbuch der landwirthschaftlichen Bakteriologie*, p. 576. Borntraeger, Berlin.

(15) MICHAELIS, L. (1911). Die Saure-Agglutination der Bakterien. *Deut. med. Wochenschr.* 37, Jahrg. I, s. 969.

(16) PEARSALL, W. H. and PRIESTLEY, J. H. (1923). Meristematic Tissues and Protein Iso-electric Points. *New Phyt.* XXII, No. 4, 185.

(17) PHILLIPS, T. G. (1920). Chemical and Physical Changes during geotropic Response. *Bot. Gaz.* LXIX, 168.

(18) PRIESTLEY, J. H. and ARMSTEAD, D. (1922). Physiological Studies in Plant Anatomy. *New Phyt.* XXI, No. 2, 72.

(19) SMITH, E. F. (1910). A new Tomato Disease of economic Importance. *Science*, N.S., XXXI, No. 803, 794–6. Also *Bacteria in relation to Plant Diseases*, 1914, III, 161. Gibson Bros., Washington.

(20) SMITH, E. F. (1911). *Bacteria in Relation to Plant Diseases*, II, 335. Gibson Bros., Washington.

(21) WAGNER, J. R. (1916). Hydrogen-Ion concentration und natürliche Immunität der Pflanzen. *Cent. Bakt. Parasitenk.* II, Abt. 44, p. 708.

(22) WALPOLE, G. S. (1914). Notes on regulator Mixtures, recent Indicators, etc. II. *Biochem. Journ.* VIII, 628.

(23) WOLF, F. A. and SHUNK, I. V. (1921). Tolerance to Acids of certain Bacterial Plant Pathogenes. *N. Carolina Agr. Expt Station, Tech. Bull.* No. 20.

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THE EFFECT OF IODINE ON SOILS AND PLANTS

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(With 8 Text-figures.)

The occurrence of small quantities of chemical elements in plants, other than those usually regarded as nutritive in function, has been attracting an increasing amount of attention of late years, and theories have been advanced as to the functions of these elements in the economy of the plant. Leading on from this, investigations have been made as to the effect of these same elements when added to the substratum or nutrient medium upon which the plants are grown, especially from the point of view of the improvement of agricultural crops. Among these elements iodine has its place, and during recent years has been investigated from both the physiological and agricultural standpoints.

Several workers claim to have obtained improved growth of crops by the use of dressings of iodine. In view of the fact that small quantities of the element occur in such artificial manures as nitrate of soda and also in kelp an attempt has been made at Rothamsted to determine whether iodine can profitably be used in agricultural practice as a partial sterilising agent or as an accessory fertiliser. Both lines of investigation have been followed up simultaneously, a grant in aid being given by the Chilean Iodine Committee, which enabled the work to be carried out more thoroughly and over a longer period than would otherwise have been possible.

A. Historical.

(*a*) *Distribution.* Chatin (4) was one of the earliest workers on the distribution of iodine, and in the vegetable kingdom he detected its presence in the fossil remains of plants, in certain aquatic and terrestrial plants and in such fermented liquors as wine, cider and perry.

Stanford (18) analysed a number of marine plants and his figures indicate that deep-sea algae usually have a greater proportion of iodine than those that grow in shallow water. Amongst others he gives the following:

Deep sea	Percentage of Iodine	Shallow water	Percentage of Iodine
Laminaria digitata (A)	0·4535	Fucus serratus	0·0856
„ „ (B)	0·2946	„ nodosus	0·0572
„ saccharina	0·2794	„ vesiculosus	0·0297

Scurti (17) investigated the iodine content of marine algae from the physiological point of view, using *Sargassum linifolium* and *Cystoseira discors* as subjects, and concluded that iodine holds in the algae the place held by chlorine in the phanerogams, its function being that of an excitant of the reproductive phase. The iodine content was found to increase regularly from the beginning of the reproductive period to the beginning of maturation of the organs of fructification, and then to decrease with completion of maturation.

Gautier (8) carried the matter of iodine distribution further, his experiments indicating that iodine is a constant constituent of green algae, both marine and those of fresh water, though less is present in the latter. Certain sulphur bacteria contain iodine, but the element is an inconstant constituent of fungi and colourless algae. Gautier concluded that in green algae the iodine either enters into the constitution of the chlorophyll itself or into that of the protoplasm concerned with assimilation, occurring in the form of a nucleinic combination rich in both phosphorus and iodine. Hence it would appear to be essential for green algae, but merely supernumerary for such plants as fungi or tobacco in which it may or may not be present.

Cameron (3) found iodine in all green, brown and red algae examined, the quantity varying from 0·002–0·2 per cent., most occurring in *Laminaria*. Analyses of different parts of *Nereocystis lutkeana* showed marked differences, but in no definite direction, indicating no specific distribution of iodine in the plant. He suggests that iodine is an almost invariable constituent of all organisms, the amount depending on the diet and the medium of the organism. Winterstein (27) analysed thirty-five phanerogams and a few fungi, but only detected iodine in small amounts in beets, celery roots, lettuce, carrot and potatoes, about 0·002 per cent. occuring in the latter.

(*b*) *Effect on plant life.* Iodine has rarely been considered to be an essential element for plant nutrition, but Mazé (13, 14) claims that minute quantities of iodine, together with boron, aluminium and fluorine, are indispensable to the growth of maize in the same sense as nitrogen, phosphorus, potassium, etc. Experiments at Rothamsted (unpublished) do not altogether bear this out, as excellent plants have been obtained in water culture in the absence of iodine, but further tests are needed.

The more usual aspect from which iodine is considered is that of its toxic or stimulating action. Voelcker (25) found that sodium iodide exercised a very harmful effect upon the germination and growth of such crops as barley, mustard, wheat, clover and peas when applied at the rate of 5 cwt. per acre, the results with lighter dressings varying somewhat with the season and crop, though they were usually unfavourable. Soaking the seed in 1 per cent. solution of sodium iodide for twenty minutes before sowing occasionally seemed slightly beneficial. Heckel (11) found that iodine hastened the germination of *Raphanus raphanistrum*, while Moore (15) obtained stimulation of the same plant in both water and soil cultures, the optimum concentration appearing to be about 1½ lbs. per acre. Japanese investigators, Aso and Suzuki (1), Uchiyama (23, 24) claim that small doses of iodine applied as potassium iodide stimulate the growth and production of various agricultural crops as rice, oats, barley, radishes, peas, sesamum and spinach, the optimum dressing varying from 25–500 grm. per hectare, and they suggest that the manurial value of seaweed may be due in some part to the iodine contained.

The efficiency of iodine as a fungicide appears to be doubtful, as Lodeman (12) sprayed various fruits with iodide of potassium solution, and found that, especially with quince, the injury caused to the foliage obscured its possible fungicidal action. In another direction Cooper and Walling (5) had some measure of success in destroying the larvae of blowfly by means of iodine vapour, 78 per cent. mortality being obtained when air saturated with iodine vapour was drawn over the larvae for 3½ hours.

B. IODINE AS AN AGENT FOR PARTIAL STERILISATION.

Several soils of varied history were obtained from farm and greenhouse, some of the latter being definitely classed as "sick" and therefore more likely to show response to the action of a partial sterilising agent. After treatment with various forms and strengths of iodine or other substances to act as controls, bacterial counts on Thornton's count medium (22) were made at 5 or 10 days intervals over periods extending from 10 days to 16 weeks. In the earlier experiments the soils were placed in the usual way in bottles plugged with cotton wool and kept at room temperature in a laboratory cupboard. Later on, in order to approximate more closely to the conditions to which partially sterilised soils are subjected in greenhouse cultures, the treated soil was placed in new unglazed earthenware pots, coated with pure white paraffin wax inside and out to prevent undue evaporation or any interaction between the porous pot and the soil. Each pot was weighed when filled, and as far as possible kept at this weight throughout the experiment by the addition of distilled water, in order to maintain a fairly constant moisture content. The actual water content in each case depended upon the initial moisture content of the soil used, as a certain amount of extra water had to be added to carry the solutions of iodine and other substances. The pots were kept in the greenhouse and were therefore subjected to the fluctuations of temperature normal to such conditions. With every soil one or two strengths of I in NaI solution were tested against untreated controls and in certain instances sodium iodide, iodoform dissolved in alcohol or ether, and cresylic acid were investigated in addition. An account of each soil and the results obtained therefrom is given to provide the data upon which the final conclusions are based. As iodine is so sparingly soluble in water and it was desired to have a certain amount of uncombined iodine in the dressings used, 12 grm. iodine with 18 grm. sodium iodide were made up to 2·724 litres with distilled water, giving a stock solution containing 1 grm. iodine per 100 c.c., partly in the elemental and partly in a combined state. This solution in appropriate dilutions was used throughout except where otherwise indicated.

1. *Unmanured soil from Little Hoos Field, Rothamsted*, as used in the pot culture experiments (see pp. 99–101).

Moisture content after treatment = 18 per cent.

A preliminary trial was made with five strengths of iodine, as I in NaI, from ·00095–·019 grm. I per kg. soil, to correspond fairly closely with the amounts received by the pot cultures running at the same time. Plates were poured 5 to 15 days after treatment, and counted 10 days later. The counts suggested that at first a considerable depression was caused by the iodine treatment, being more marked with the smaller doses, but that after a longer interval the bacterial numbers, with the heavier dressings especially, rose considerably above the untreated controls (fig. 1). This was repeated with the heaviest and lightest dressings (·019 and ·00095 grm. I), pourings being made one day after treatment and then at five successive five-day intervals. The initial depression with iodine five days after treatment was again obtained, but the later rise above the control was not obtained, the difference in the counts being within experimental error (fig. 2).

2. *Old cucumber soil* from Mr W. B. Randall, Harpenden.

This soil had been turned out of the greenhouse for some time and

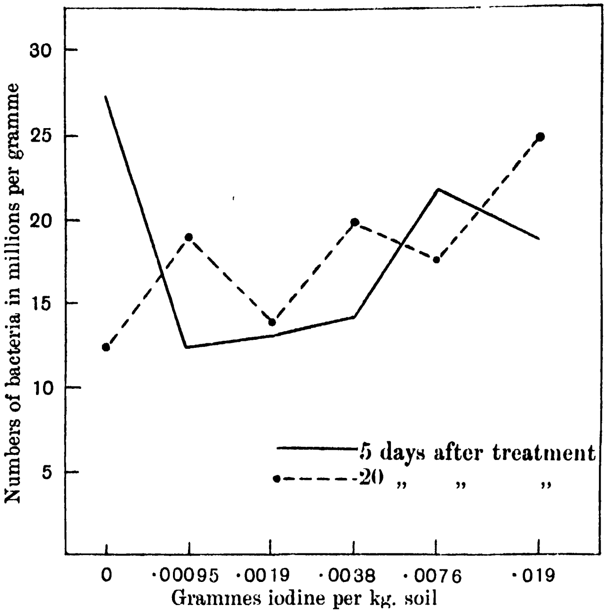

Fig. 1. Bacterial numbers in Little Hoos soil, Rothamsted (1), after treatment with various strengths of iodine.

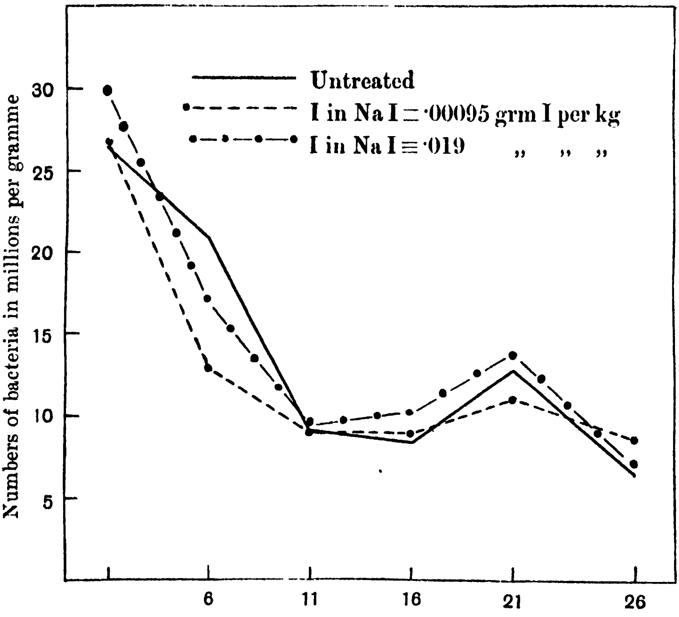

Fig. 2. Bacterial numbers in Little Hoos soil (1), after treatment with iodine.

exposed to the weather, and was consequently very moist owing to the large quantity of organic matter present, though it was friable, sifted easily and did not cake when worked.

Moisture content after treatment = 43·95 per cent.

Tested: I in NaI = ·019 and ·0038 grm. I per kg. The waxed pot method was used in this test and henceforward.

The heavy dressing of iodine had an immediate effect in considerably raising the bacterial numbers, by 25 millions per grm. over the control which gave 87·5 million per grm. This increase was maintained for ten days and then disappeared, the lower dressing having no significant effect throughout (fig. 3).

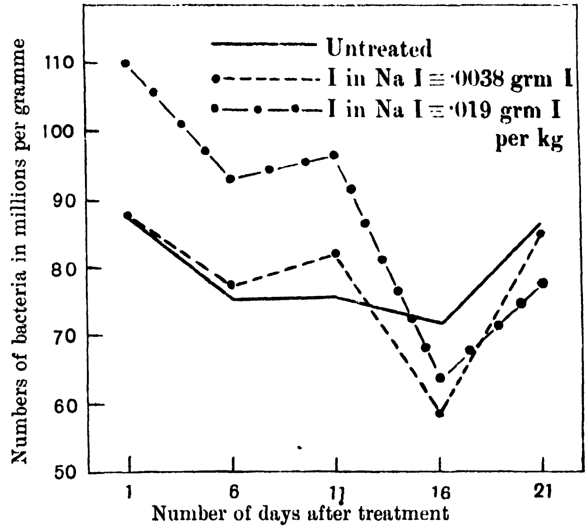

Fig. 3. Bacterial numbers in old cucumber soil, Harpenden (2), after treatment with iodine.

3. *Tomato sick soil*, from Mr Matthews, Shrewsbury.

This soil has had tomatoes grown in it for 20 years and has not been treated at all in the way of partial sterilisation, either by steam or chemicals. The plants are now doing very badly and the season before receipt (May 1923) the grower had had to replace fifty per cent. of them.

Moisture content after treatment = 21·4 per cent.

Tested: I in NaI = ·019 and ·0076 grm. I per kg. soil.
Iodoform = ·0076 grm. I per kg. soil.
Cresylic acid = ·25 per cent.

As an aqueous solution of iodoform could not be made, a weighed amount of iodoform was dissolved in as little alcohol as possible and the solution very thoroughly mixed with fine dry sand in such quantity that 10 grm. of the treated sand would contain the requisite amount of iodoform for one pot. The sand was spread out on a clean sheet of glass to allow the alcohol to evaporate, and then 10 grm. was intimately mixed with the weighed amount of soil for each pot. To keep the treatment uniform 10 grm. of similar sand, but untreated, was added to each of the other pots in the series. Cresylic acid was used in this experiment to provide a contrast with an agent with known powers of partial sterilisation, the amount applied corresponding to one of the heavier dressings used in commercial practice. Counts were made at five-day intervals for three weeks and an additional set of pots was carried over for 16 weeks before pouring, to ascertain if any effect were noticeable after the lapse of some considerable time from treatment. With this soil the I in NaI proved ineffective throughout with both dressings, the curves of bacterial numbers running close and fairly parallel to that of the untreated control. Cresylic acid showed the expected drop directly after treatment, followed by a steady rise, until after three weeks the numbers were approaching those of the untreated and iodine soils. The dressing of cresylic acid would appear to have been rather too heavy, for the anticipated large rise in bacterial numbers, after the initial fall, had not begun to show itself three weeks after treatment. As no counts were made between July and October, it remains uncertain whether the rise ever manifested itself. An interesting point occurred in the October counts, as every plate from cresylic acid treated soil was filled with a heavy growth of mould, which did not appear in any of the controls or iodine or iodoform plates. This suggests some encouragement given to moulds by cresylic acid, and might be worth following up in view of the variable results sometimes obtained when this agent is used for partial sterilisation by market gardeners.

The soils dressed with iodoform manifested a striking increase in bacterial numbers five days after treatment, succeeded by a similar rapid fall within ten days more to the level of the controls (fig. 4). This rise to 325 millions per grm., compared with the control 50 millions, can hardly be fortuitous, though no adequate explanation is as yet forthcoming. The iodoform was carried in alcohol as a solvent, which was evaporated before application to the soil. Under these circumstances, if the iodoform were the active agent, the effect should be reproduced when other solvents, such as ether, were used, but further tests (see 4

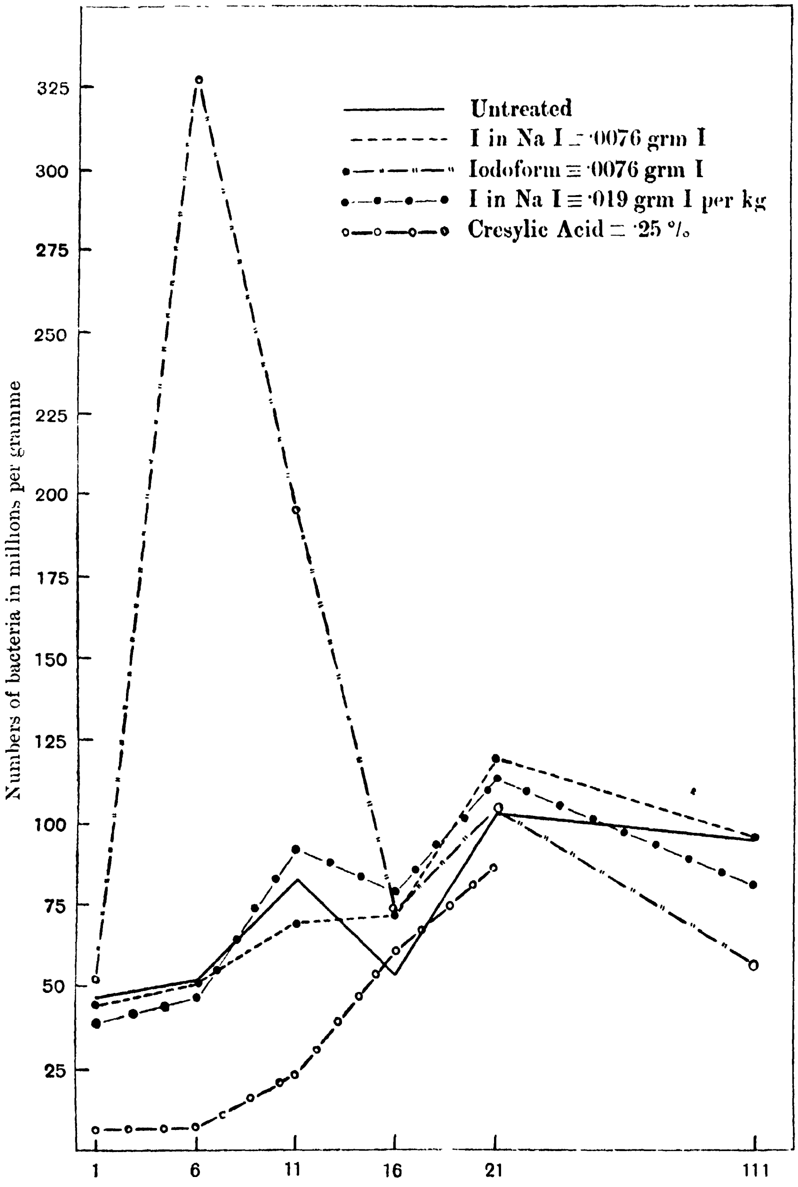

Fig. 4. Bacterial numbers in tomato-sick soil from Shrewsbury (3) after treatment with iodine or other reagents.

and 5) did not seem to bear this out to any extent. Suspicion was directed to the purity of the alcohol, which was the ordinary stock alcohol stored in metal drums. The quantity used amounted to about 1 c.c. for each 10 grm. sand, *i.e.* for each pot treated. It is inconceivable that any residue that may possibly have been left by this small amount of alcohol should contain sufficient bacterial food, presumably nitrogenous in nature, to provide nutriment for such a large number of organisms. So one is faced with the remaining proposition that the alcohol left a residue which in some unexplained way greatly stimulated the division of bacteria for a short time after application, the effect wearing off rapidly after the first few days. This point could not be followed up immediately as no more of the soil was available, but it is hoped that further supplies may shortly be obtained enabling more extended tests to be carried out. At the end of three months less bacteria were present in the iodoform soils than in the others, but no weight can be placed on this isolated result.

As the effect of partial sterilisation is shown not only by rise in bacterial numbers, but also by increase in the amount of nitrate and ammonia present in the soil, the quantity of these was estimated at each pouring for every treatment in the experiment (Table I). The normal course of events was illustrated with cresylic acid, in which the ammonia rose from 2·24 p.p.m. to 13·4 p.p.m. in 15 days, and then fell off, being at the original level again when examined after 16 weeks. The untreated controls showed no ammonia on any occasion, and very little was present with either strength of I in NaI except on two occasions with the heavier

Table I. *Nitrogen in parts per million in soils on days bacterial plates were poured.*

N as NH_3

Before treatment, June 6th, 0·56 p.p.m.

	June					Sept.
	7th	12th	17th	22nd	27th	25th
Control	nil	nil	0·56	nil	nil	failed
·0076 grm. I ...	0·56	0·12	0·56	1·12	1·12	0·89
·019 „ ...	1·12	0·56	3·35	1·68	1·12	3·13
Iodoform ...	4·24	2·24	1·12	1·12	1·12	0·89
Cresylic acid ...	2·24	7·81	10·05	13·40	10·95	2·12
	N as N_2O_5					
	Before treatment, June 6th, 121·1 p.p.m.					
Control	122·3	121·1	122·1	120·6	77·20	15·15
·0076 grm. I ...	115·9	125·3	125·0	110·3	60·38	12·56
·019 „ ...	119·9	125·3	123·6	117·3	62·30	19·55
Iodoform ...	118·6	109·4	111·6	115·9	73·76	48·82
Cresylic acid ...	120·1	113·8	116·3	111·9	109·20	21·64

dressing when a slight increase occurred. In view of the bacterial rise with iodoform, however, it may be significant that 1 p.p.m. ammonia was present in the soil one day after treatment, though this amount fell within 10 days to the level of the I in NaI soils and remained there throughout.

The nitric and nitrous nitrogen of the untreated soils remained very constant for the first 15 days, fell rapidly during the next five days, and by the end of 16 weeks was down to only 15 p.p.m. instead of 122 p.p.m. at the beginning. With the various iodine treatments slight fluctuations, which are of doubtful significance, occurred during the first 15 days, followed by a similar fall to that with the control, though at the end of 16 weeks the iodoform soil still contained far more than any of the others. The rapid fall in nitrogen as N_2O_5 between June 22nd and 27th is difficult to understand, as no plants were growing in the soil, but duplicate tests were made to prove the accuracy of the analyses. With cresylic acid the beginning of the fall was delayed, but ultimately the amount approximated to that in most of the other soils.

The failure to obtain definite partial sterilisation effects with iodine, as evidenced by increase in bacterial numbers, would therefore seem to be borne out by the estimations of nitrogen in the soils, the only suggestive correlation being that of the initial rise in bacterial numbers and increase of ammonia in soil treated with iodoform.

4. *Tomato soil from Mr Matthews, Shrewsbury*, which had carried tomatoes for six years.

Moisture content after treatment = 19·55 per cent.

Tested: I in NaI = ·0076 grm. I per kg. soil.
Sodium iodide = ·0076 ,, ,,
Iodoform in ether = ·0076 ,, ,,
Ether alone 1 cc. per pot, evaporated off

In view of the possible interaction of alcohol residues in the iodoform treatment the reagent was dissolved in ether, mixed with sand and evaporated off as before. A further check was applied by treating soil with ether alone, to find out whether any action occurred therefrom, any difference between the two series being presumably attributable to the iodoform.

No evidence of partial sterilisation could be deduced from the bacterial counts, though for some days the numbers with iodoform were considerably above those of the controls. Nevertheless the curves run so very parallel in direction, and the differences in number between one treatment and another are so slight that they cannot be regarded as significant (fig. 5).

No repetition whatever was obtained of the great rise in bacterial numbers with iodoform at the beginning of the test, leaving it still an open question as to whether this previous rise was due to the alcohol used as a carrier. Throughout the experiment the iodoform soil was richer in bacteria than that receiving ether alone, especially towards the end, and on some dates the difference was sufficiently great to suggest a true increase arising from the use of iodoform. This soil is very poor in bacteria, only 3·7 millions per grm. being present before treatment, as compared with 8·6 and 10·0 million per grm. in soils 3 and 5 respectively. Examination for protozoa revealed a great paucity in the number of species, both actually and in comparison with those in the other two soils.

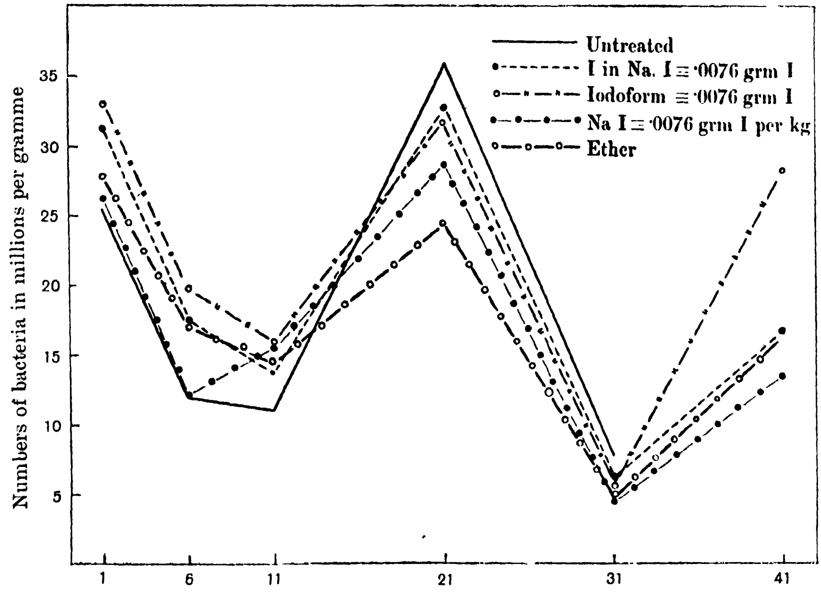

Fig. 5. Bacterial numbers in tomato soil from Shrewsbury (4) after treatment with iodine.

5. *Tomato soil from Mr J. C. Randall, Waltham Cross,* which had carried tomatoes since 1919 and had not been partially sterilised. The house from which the soil was taken was a poor one as regards cropping, but this may have been due to defective heating rather than to "sickness" of the soil.

Moisture content after treatment = 26·763.

The tests were repeated as under (4), except that the equivalent amount of iodine was ·019 grm. per kg. soil. In this case the relative numbers of bacteria were less regular than in any of the previous tests, the curves of the counts being much less parallel (fig. 6). The irregularities were such, however, that no evidence was obtained of any constant or definite effect of any of the dressings employed, and again the iodoform carried in ether did not show the rise obtained with alcoholic iodoform.

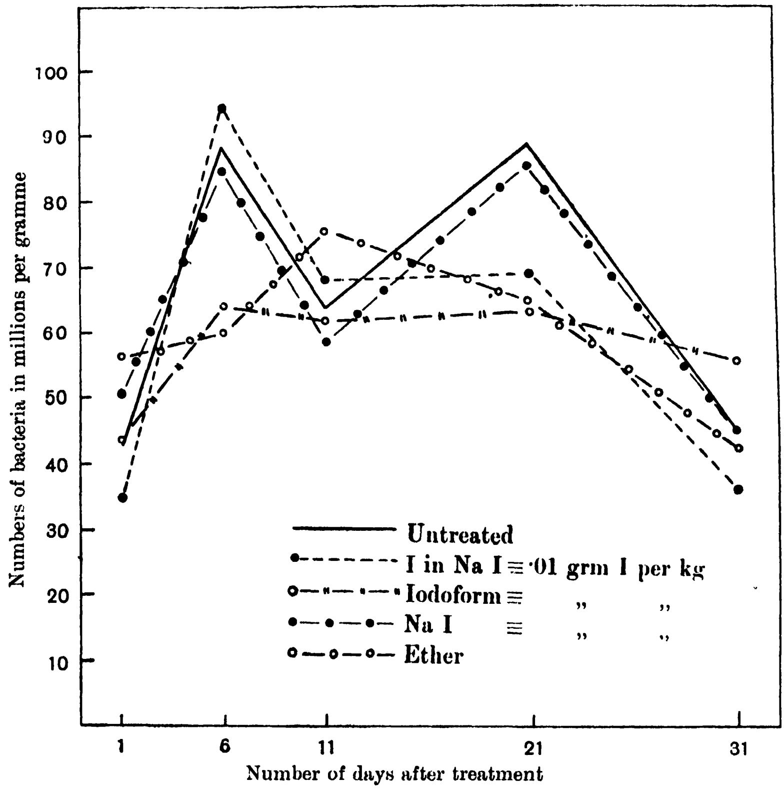

Fig. 6. Bacterial numbers in tomato soil from Waltham Cross (5) after treatment with iodine.

The results set forth above have been obtained from a consideration of various soils after treatment with iodine presented in different combinations and concentrations. In some cases the effect produced by the iodine, even if fairly constant, was too small to be regarded as of any significance, if, indeed, it was not within the limits of experimental error. In other cases, in which iodine occasionally produced considerable increase or decrease in bacterial numbers the variations were too irregular and inconstant to indicate any definite partial sterilisation brought about by the iodine, the bacterial numbers in the treated soils ranging above or below those of untreated soils at different times. The only very great increase lies under suspicion on account of the possible impurity of the alcohol carrier, but is sufficiently outstanding to justify further work if the necessary soil can be obtained.

C. EFFECT OF IODINE ON GERMINATION AND "DAMPING OFF" OF SEEDLINGS.

In order to gain some idea whether iodine dressings would improve the germination and early growth of tomatoes in reputed "tomato sick" soil, glazed pots holding 5 or 6 lbs. were filled with the soil from Shrewsbury and Waltham Cross used in tests (3) and (5) above, one-third being left untreated, and the others dressed with ·019 and ·0076 grm. I per kg. soil, applied in the usual solution of iodine in sodium iodide. In order to give any partial sterilisation time to take place sowing was delayed for three weeks, except with some of the Shrewsbury pots which were sown after 10 days. During the interval the pots were kept moist, and then each received seven seeds of Sutton's Early Market, duplicate or triplicate sowings being made for each treatment. In no case did any obvious difference appear either in the rapidity of germination or in the initial vigour of the seedlings in the treated and untreated pots, but after three to five weeks, according to the date of sowing, the plants in the iodine-treated soils were rather taller and had formed larger and better leaves. At this stage the seedlings were thinned out to 1 per pot, and for some time after the plants in the untreated soils remained behind the others in development in the Shrewsbury soil, whereas in the Waltham Cross soil they soon took the lead. As the experiment was only designed to test the effect on germination and small pots were used in consequence, it was soon necessary to feed the plants rather heavily, and any initial effect due to the iodine treatment was swamped in consequence. As a matter of interest a record was made of the amount of fruit produced per plant, but no practical results issued therefrom.

In yet another connection experiments were carried out by Dr Bewley, at Cheshunt, to determine whether treating the soil with iodine before sowing would reduce the percentage of loss from "damping off" of seedlings. The same stock solution was used as at Rothamsted, iodine in sodium iodide containing 1 grm. I per 100 c.c., and the normal solution used in the tests was 1·2 c.c. of the stock in 10 ozs. water. Tests were made with quantities varying from 5 to ⅕ normal, against controls receiving distilled water only, and with each concentration two boxes containing seven pounds of soil received 10 ozs. of the appropriate solution, afterwards being sown with 100 seeds apiece. The percentage of germination was not affected by any of the treatments and the variation in the percentage of diseased seedlings bore no relation to the amount of iodine used, no benefit being thus derived from the application (Table II). With five times normal solution germination was retarded and the shoots were dark green, but ultimately the percentage results resembled those with all other dressings.

Table II. *Effect of Iodine on "damping off."*

Treatment	% germination	% diseased
5 normal	98	11
2 ,,	97	15
1 ,,	97	17
½ ,,	98	13
⅕ ,,	99	10
Control (dist. water)	97	15

D. EFFECT OF IODINE ON PLANT GROWTH.

The main experiments were carried out in pot cultures with mustard and barley, but further tests were made in water cultures with other plants. Mustard and barley are useful foils for one another in investigations of this kind, as mustard represents a type of crop that is cut green, before it has fully completed its reproductive phase, whereas barley is one of the group in which the ripe seed is the most important desideratum, and in which growth is allowed to continue until it practically ceases. It may well happen that some specific substance added to the soil may exercise a beneficial or a toxic action while vegetative growth is in full swing, but that the difference may become less marked as the plant nears the end of its life and ripens its seeds. On the other hand, the reverse may possibly occur, and the action of the specific substance only become manifest in the reproductive phase.

Experiments A. Soil treated Aug. 30th, 1922.

The initial tests were made in unmanured heavy loam in pots containing about 10 kilos (23 lbs.), to each of which was added a basal dressing of 4 grm. 35 per cent. super, 1 grm. K_2SO_4, 1 grm. $NaNO_3$, a further 1 grm. $NaNO_3$ being applied as a top dressing during growth. Iodine in sodium iodide solution was applied at the rate of 1·0, 0·2, 0·04, 0·008 grm. I per pot, equivalent to approximately ·1, ·02, ·004, ·0008 grm. I per kg. of soil. The required amounts of stock solution were made up to 100 c.c. with water and applied evenly over the surface, another 100 c.c. of water being similarly applied when the seeds were sown a day later, mustard and barley being tested. Further watering was carried out through a porous pot sunk into the soil.

Mustard. Sown Aug. 31st, 1922. Cut Dec. 19th, 1922.

Iodine exercised a distinctly harmful effect upon germination. With the heaviest dressing a few seeds chitted, but the tiny rootlets soon died and no plumules emerged, while with one-fifth the dressing a very few plants survived and developed small plumules. Even ·04 grm. I per pot considerably reduced the number of seeds that germinated, and also the vigour of the young plants, but the weakest dose, ·008 grm. I, gave a stand equal to that of the untreated control pots. The plants were thinned to three per pot, and those with ·04 grm. I gradually recovered from their initial check and ultimately resembled the controls. The most striking feature, however, was the behaviour of the plants with ·2 grm. I. At first it was uncertain whether they would live or die, the seedlings being dark, unhealthy and very poor, but after about a month growth set in in earnest, though very irregularly. Rapid improvement took place, the leaves becoming larger than usual, though the colour was pale and the texture soft and succulent, until at harvest time some of the plants appeared as good as the controls, though shorter on the average. The green weights showed a gradual rise with increase in the dressing of iodine, very little significant difference appearing in the dry weights. The percentage of nitrogen in the dry matter and the total nitrogen removed by the plants tended to increase with the iodine. This increase in nitrogen, though not great, was consistent throughout, and if corroborated by further experiments might be a matter of importance, especially if correlated with the heavier green weight, as for crops that are cut green the latter would provide increased bulk associated with higher nutritive value due to the extra nitrogen.

Table III. *Mustard. Average of six pots.*

Iodine per pot grm.	Av. height of plants cm.	Green weight grm.	Dry weight grm.	% dry in green	% N in dry	Total N grm.
Control. No. 1	68	97·21 ± 1·23	13·28 ± ·18	13·68 ± ·20	3·13	·402
0·008	72	99·31 ± 2·15	12·28 ± ·29	12·42 ± ·29	3·24	·398
0·04	65	103·98 ± 2·62	12·83 ± ·36	12·36 ± ·23	3·42	·440
0·2	58	111·56 ± 4·37	11·96 ± ·78	10·75 ± ·65	3·70	·443

Barley. Sown Sept. 5th, 1922. Cut Nov. 20th, 1922.

It soon became evident that barley is much more sensitive than mustard to the toxic action of iodine. With the heaviest dressing no sign of germination occurred, and with one-fifth the dose a few seedlings appeared above the ground, but made no further growth and soon died off without developing. ·04 grm. I per pot hindered germination and growth, but whereas with mustard the plants with this strength made a rapid recovery from the initial injury and ultimately surpassed the controls, no signs of such recovery occurred with barley, the green weight at the time of cutting being reduced to 75 per cent. and the dry weight to 56 per cent. of that of the controls. The weakest dressing, ·008 grm. I per pot, had no effect on germination or growth, both green and dry weights tallying remarkably well with the controls. There was thus no evidence of any beneficial or stimulating action in this instance with barley. The crop had to be cut before ears were formed as it was grown late in the year and danger threatened from mildew.

Table IV. *Barley. Average of six pots.*

Iodine per pot grm.	Green wt. grm.	Dry wt. grm.	% dry in green	% N in dry	Total N grm.
Control. No. 1	40·36	5·38	13·31	5·13	·276
0·008	40·78	5·28	12·96	5·21	·276
0·04	30·26	2·99	9·85	5·78	·173
0·2	—	—	—	—	—

The percentage and actual nitrogen present in the crop were unaffected by the smallest dose of iodine, and though the percentage of nitrogen was higher with a dressing of 0·04 grm. I per pot the total nitrogen present was considerably less than in the controls, affording no comparison in this respect to the mustard plants treated to correspond.

As the gradual recovery of retarded mustard plants in the parallel experiment indicated a reduction in the toxicity of iodine dressings as time went on, barley was sown again three weeks after the soils had been treated in the pots in which it had previously failed, *i.e.* with 1 grm. and 0·2 grm. I per pot respectively. All the seeds germinated within a week, but while those with 0·2 grm. I made good growth and did not seem at all adversely affected at any stage, those with 1·0 grm. I were small from the beginning and made very little growth, eventually dying off in about six weeks. This provides clear evidence of the reduction in toxicity with time, a reduction that was practically complete with the lighter dressing, but with the heavier dressing tried was not sufficiently great to permit normal growth, though distinct improvement was effected, as shown by the fact that the seeds germinated and made some growth in soils in which originally no signs of germination occurred.

Experiments B. Soil treated Feb. 27th, 1922.

In order to determine how rapidly and completely the toxic effect of heavy doses of iodine would pass off, and also to find out if the toxic action would then be replaced by a beneficial one, a series of sowings of barley and mustard were made in pots treated on the same day. The soil was manured as in Exp. A, except that 2·5 grm. sodium nitrate was supplied per pot for mustard, 2 grm. for barley, two series of pots being treated with light and heavy dressings of iodine in sodium iodide solution, a third series being left untreated. Three pots of each were sown the day after treatment and the rest at seven-day intervals over a period of five weeks.

About eleven weeks after the soil was treated with iodine additional sodium nitrate was applied as a top dressing dissolved in water, 1·0 grm. per pot to mustard, 0·5 grm. per pot to barley.

Mustard. Sown Feb. 28th, 1922, and at weekly intervals. Harvested June 18th.

When sown *one day after treatment* germination was affected as in the previous experiment, the plants with the light dressing recovering fairly soon, those with the heavy dose remaining small and irregular for several weeks, after which they began to grow very vigorously, ultimately surpassing the others in height and stoutness of stem. Flowering, however, was delayed by treatment by about a week with the heavy doses. The green weights showed the same rise with iodine as before, the dry weights being fairly level, with a possible increase with heavy iodine.

With a delay of *one week after treatment* before sowing germination was still affected with both doses of iodine, but recovery was more rapid, and within a fortnight the treated plants were as large as those sown a week earlier, indicating that the harmful action had been in some degree mitigated by the delay. The same retardation of flowering occurred with the heavy iodine dressing, but the plants at harvest time looked quite as good as the controls, though more succulent. The green and dry weights showed a considerable increase over the controls, and also the percentage of dry matter in green was rather higher. The light dressing of iodine, however, did not yield similar increases, the figures not being significantly different from those of the controls. As the period of delay before sowing was prolonged the check to germination caused by iodine decreased, until after four weeks it was hardly noticeable. With the improved germination less initial injury was manifest and the recovery therefrom was less marked, apparently not being followed by a corresponding stimulation of growth as would have been the case had the toxic effect of iodine given way to a beneficial action. Statistical examination of the weights of the plants and consideration of the experimental errors involved show that there is no significant difference between

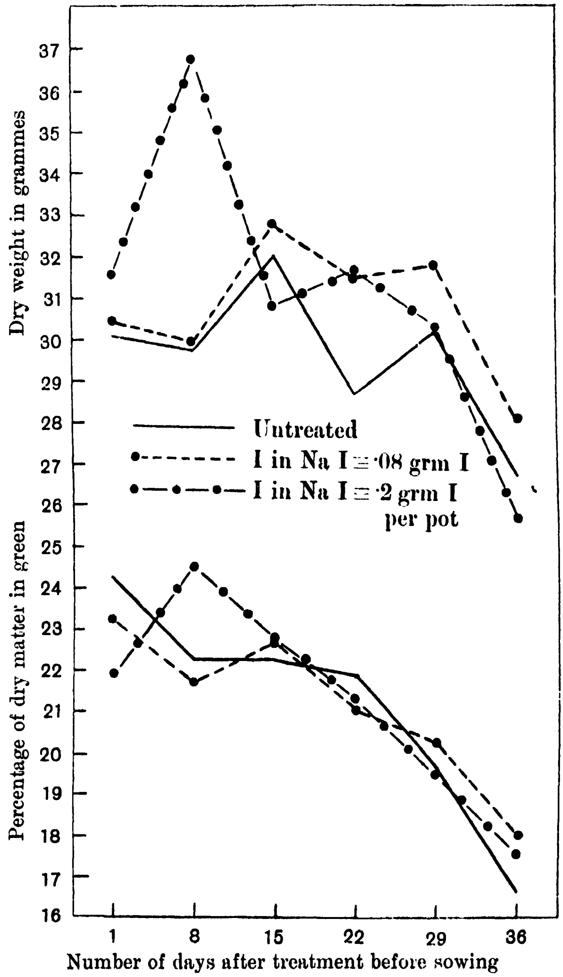

Fig. 7. Dry weight and percentage of dry matter in green in mustard sown at varying periods, after treatment of the soil with iodine.

the control and iodine treated plants except in the case of the rise with the heavy dressing after one week's delay before sowing (shown by figures in black type in Table V). The general fall in the dry weight shown in the curve (fig. 7) is due to the decreasing age of the plants, the

Table V. *Mustard. Average of three pots.*

Treatment (iodine in grm. per pot) and time of delay before sowing	Green wt. grm.	Dry wt. grm.	% dry in green	% nitrogen	Actual nitrogen present av. per pot
1 *day*					
Untreated	124·26	30·08	24·23	1·645	·495
0·08	130·89	30·42	23·26	1·640	·499
0·20	144·08	31·54	21·94	1·875	·591
1 *week*					
Untreated	133·80	29·75	22·24	1·647	·490
0·08	137·7	29·97	21·71	1·807	·542
0·20	**150·3**	**36·72**	**24·47**	1·568	·576
2 *weeks*					
Untreated	142·98	31·99	22·43	1·650	·528
0·08	144·67	32·77	22·64	1·603	·525
0·20	135·53	30·80	22·72	1·827	·563
3 *weeks*					
Untreated	130·83	28·64	21·88	1·675	·480
0·08	149·62	31·43	21·04	1·644	·517
0·20	149·65	31·68	21·30	1·748	·554
4 *weeks*					
Untreated	153·72	30·15	19·65	2·002	·604
0·08	156·96	31·77	20·24	1·927	·612
0·20	155·77	30·32	19·50	1·945	·586
5 *weeks*					
Untreated	160·92	26·68	16·64	2·098	·560
0·08	156·48	28·07	17·99	2·127	·597
0·20	144·77	25·65	17·56	2·111	·535

corresponding rise in the green weights (Table V) being associated with the greater degree of desiccation that had occurred in the older and more mature plants, the combination of the two factors being reflected in the almost steady fall in the percentage of dry matter in green, only broken seriously by the marked rise with the heavy treatment sown after a week's delay. The percentage of nitrogen present in the dry matter of the plants varied somewhat at first with the treatment, but not in any definite direction, though it showed a slight tendency to be higher with the heavy dressing of iodine. After about three weeks' delay before sowing the percentage rose somewhat and was practically the same with every treatment. The actual amount of nitrogen absorbed by the plants was greater with the larger dose of iodine for the first four sowings, the increase tending to get less as the time of sowing was more delayed. After this the tendency appeared to be in the other direction, but the irregularity of the dry weights at the last sowing indicate that little significance can be attached to this point. The consistent increase of nitrogen with heavy dressings of iodine at four successive sowings suggest that the iodine has some influence upon the absorption of nitrogen in the plant, which persists for a longer time than the influence upon the dry weight production.

The extra nitrogen supplied to the soil as nitrate of soda was 0·5765 grm., and with the earlier sowings it rarely happened that the nitrogen present in the plants amounted to as much as that except with heavy iodine dressings, showing that an ample supply of nitrogen was available, as the equivalent of the original store in the soil remained untouched. With the two latest sowings, however, nitrogen absorption was considerably greater, being usually above the amount presented as nitrate of soda.

If, therefore, iodine exercises any true beneficial action on the growth of mustard, it is to be looked for when a heavy dressing is applied and sowing is delayed for about a week, when a somewhat irregular and abnormal crop is to be expected, subject to the danger of loss if adverse conditions should prevail when the plants are very young and still suffering from the check due to the preliminary toxic action of iodine. The increase of nitrogen content continued after this period might be advantageous under certain circumstances, but is probably too slight to be of any real significance.

Barley. Sown Feb. 28th, 1923, and at weekly intervals. Harvested Aug. 8th, 1923.

When sown *one day after treatment* germination was delayed for a few days with the heavier dose of iodine, and when the plants did appear they remained very small and were of an unhealthy dark green colour. Little progress was made, and within three weeks all were dead. With the lighter dressing germination was not hindered and at first the seedlings were comparable with those in untreated soil, but they rapidly fell behind, becoming unhealthily dark in colour, and developing few tillers. After a few weeks several of the plants died and in no case was a recovery made similar to that noted with mustard sown the same day, with parallel treatment. As the fructifying period approached iodine was found to retard the development of fruiting stalks by about five days, and fewer ears were ultimately developed than in the untreated pots. The differences in maturity were hardly noticeable when the plants were harvested as they were allowed to remain uncut until practically the whole series had reached the stage of ripeness. An interesting point is that in spite of the early injury to the plants caused by iodine and although they failed to recover and looked more or less unhealthy to the end, yet the green and dry weights of the straw and ears were very similar to those of the untreated plants, the differences being insignificant on statistical examination.

With a delay of *one week after treatment* before sowing the heavier dose of iodine proved somewhat less toxic than before, as germination was fairly good and the seedlings developed better for some time. They gradually fell behind, however, and within six weeks nearly all were dead, only a few small weakly plants struggling to maintain a hold on existence. At harvest time, when plants were dead ripe, the few remaining plants with iodine were simply bunches of green leaves with a solitary green ear in one case. The weaker dose of iodine appeared to have little harmful effect on the vegetative growth as the plants were nearly, if not quite, as good as the untreated controls. The development of fruiting stems was rather irregular and somewhat retarded, but this difference soon disappeared.

With a delay of *two weeks after treatment* a slight retarding action was still noticeable with the heavier dose of iodine, but with this exception in all the sowings the toxic effect of iodine disappeared entirely and was not replaced by any form of stimulation. The difference between the dry and green weights of the straw and ears with the different treatments on each occasion was statistically insignificant, and as all the sets were allowed to ripen before any were cut the total dry weights were very similar whatever the time of sowing (fig. 8) though the dry weights of the ears tended to be rather heavier with the earliest sowing. The considerable drop in green weight in the last sown plants is noticeable, but has no apparent significance. The reduction was entirely in the straw, the ears at the same date being quite as heavy as on previous occasions.

The percentage of nitrogen present in the straw tended to rise in the presence of iodine, whether in strong or weak doses, but no regular effect was observed in the ears, though a slight tendency towards an increase with iodine was manifest. With the control plants the nitrogen percentage increased in both shoots and ears for the first four sowings and then dropped considerably. In the shoots weak iodine caused some rise in nitrogen percentage as time went on, but strong doses gave quite irregular results. In the ears the percentage remained fairly constant with both dressing suntil the last sowing, when a distinct fall occurred (Table VI).

The actual amount of nitrogen taken up by the plants varied somewhat, but quite irregularly, both with the dressing of iodine and the date of sowing, but the amount in the iodine-treated plants was never less than in the controls and tended to be higher.

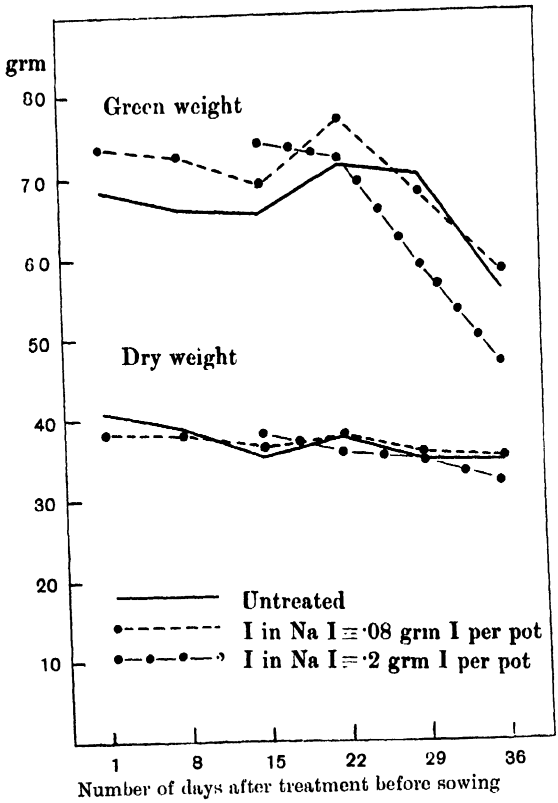

Fig. 8. Green and dry weights of barley, sown at varying periods after treatment of the soil with iodine.

Table VI. *Barley. Average of three pots.*

Treatment (iodine in grm. per pot)	1 day	1 week	2 weeks	3 weeks	4 weeks	5 weeks
		Percentage of nitrogen				
			Straw			
Control	·658	·633	·756	·857	·783	·752
0·08	·823	·816	·782	·823	·936	·911
0·20	—	1·800	·725	1·000	·890	·909
			Ears			
Control	1·64	1·70	1·99	2·00	1·92	1·66
0·08	1·76	2·03	2·08	2·15	2·22	1·65
0·20	—	—	2·00	2·21	2·03	1·86
		Nitrogen present per pot				
	grm.	grm.	grm.	grm.	grm.	grm.
Control	·428	·400	·406	·460	·406	·380
0·08	·450	·450	·428	·457	·471	·416
0·20	—	—	·441	·479	·433	·396

E. General Discussion.

The rapid loss of the effective action of iodine may be due to the element being in some way removed from the sphere of action of the plant roots. Investigations have shown that iodine is adsorbed by various substances, Scheringa (16) and other workers proving its adsorption by sand, silica, opal, agate, alumina, magnesia, beryllia and charcoal. Suzuki (19) showed that iodides are more readily absorbed by soil than chlorides. It is thus possible that part of the iodine applied to the soil may soon be rendered inactive by adsorption from solution by various constituents of the soil. This, however, would not account for the complete removal of iodine, as an adsorption equilibrium would be reached, at which point a certain concentration of iodine would be maintained in the soil solution, any iodine taken up by plants being replaced by a further supply derived from that originally adsorbed. A more probable cause of loss of iodine in its elemental state may be found in its very reactive properties and its proclivity to form addition or substitution products with various compounds occurring in the soil. Unsaturated fatty acids, such as oleic acid, can fix a number of atoms of iodine or other halogens equal to the number of hydrogen atoms necessary to saturate the molecule (Droop-Richmond (6), Grandeau (9)). It is therefore possible that if certain of these compounds occur in the soil they may unite with the iodine applied and so render it ineffective and unable to exert any influence upon plant growth. Again, iodine readily acts as a substitution element on many organic compounds of the same type as those resulting from the decomposition of organic matter in the soil, such as alcohols, ketones, aldehydes, etc., and is possibly put out of action in this way. Furthermore, interaction of iodine and alkali results in the production of iodides and hypoiodides, the latter of which have powerful oxidising properties and are unlikely to remain long as such in the soil. In the latter case the elemental iodine is rapidly converted into iodides, which would appear to have little action, judging from the fact that the plants treated with iodine in sodium iodide solution are as good as the controls when sown some time after treatment, when the conversation of iodine into iodides might be expected to be complete.

This conversion into iodide may possibly have some bearing on the failure to obtain partial sterilisation effects with iodine in spite of its great power of reaction. Sodium iodide alone appears to be inactive throughout, as indicated by figs. 5 and 6. With most of the soils tested iodine in sodium iodide had little or no effect upon the bacterial numbers, but in one case, with soil 2, a definite increase occurred with the heavy dressing. This may conceivably mean that usually the iodine was so rapidly removed, possibly by conversion into iodide, that it had no time to exert any specific action, but that in the one case cited the removal was less rapid, and sufficient iodine was left for some time to act in the direction of partial sterilisation. This argument would also apply to the other means above indicated whereby iodine is removed from action, assuming it to be correct that iodides are inactive and the free iodine is an active agent.

In this connection it may be permissible to suggest that the great increase in bacterial numbers obtained in one instance with iodoform was due to the fact that this compound is in reality very potent although under most conditions it is rapidly put out of action, but that in the case in point its destruction was slow enough to allow time for it to exhibit a considerable degree of partial sterilisation. This is merely put forward as a suggestive hypothesis, but if it could be proved to be true, and if the conditions necessary to maintain the active power of the iodoform could be discovered, it might yet happen that iodine, in appropriate combination, could be turned to valuable account for agricultural and horticultural purposes.

It has been shown above that barley is very sensitive to the toxic action of iodine and that small doses, even after a considerable delay between treatment and sowing, fail to increase the crop in any way. Mustard, on the other hand, is more resistant to the toxic action, and strong seedlings that escape being killed by a relatively heavy dressing of iodine are apt to make rapid headway and to surpass untreated control plants both in green and dry weight, if sown at the appropriate time after treatment. This suggests that different species of plants vary in their reaction towards the element and consequently there is no justification to assume, without proof, that iodine has no beneficial action on any of the agricultural crops.

Previous work has shown that the absorption capacity for iodine of plants grown on the same soil varies to some extent with the natural order. Bourcet (2) tested a number of plants on soil containing 0·83 mg. iodine per 100 kilos soil and found that the iodine present in the plants when harvested varied from 0·00–0·94 mg. per kg., *Allium sativum* being the richest with 0·94 mg., Liliaceae and Chenopodiaceae absorbing iodine much more freely than Solanaceae and Umbelliferae. This may be compared with the work of Warington (26), who has demonstrated a similar variable response with regard to boron, a small amount of the element being essential for the satisfactory growth of broad bean, scarlet runner and crimson clover, whereas barley, rye, vetch and pea are able to develop fully in its absence.

It has been suggested that root crops, such as mangolds and sugar beet, benefit from the use of iodine, and field experiments might profitably be carried out with these plants, especially in view of the fact that the wild beet, *Beta maritima*, is a native of the seashore where iodine is available owing to its presence in sea water. Pot experiments would be useless in these cases, owing to the large size and great individual variation of the plants.

SUMMARY.

1. No definite evidence of partial sterilisation with iodine was obtained when a number of different soils were treated with various combinations and strengths of iodine, the bacterial numbers with different treatments varying either very little or inconsistently. A suggestion of beneficial action with iodoform was obtained, if the right conditions could be found.

2. Germination of tomato seeds in "sick" soils was not affected by treatment with iodine in sodium iodide, and the initial vigour of treated and untreated plants was very similar.

3. The loss from "damping off" of tomato seedlings was not reduced by treatment before sowing, over a wide range of concentrations of iodine in sodium iodide.

4. Germination of mustard is either inhibited or badly checked by strong doses of iodine. In the latter case some of the plants eventually make a striking recovery and ultimately surpass the untreated controls in green and dry weight. If some time elapses between treatment and sowing the toxic action of iodine disappears and the mustard is unaffected, showing neither the initial toxic effect nor the later recovery and stimulation.

5. Barley is decidedly more sensitive than mustard to the toxic action of iodine, as germination is inhibited by a smaller dressing. With an amount of iodine that allows the seeds to germinate, though checking them badly, the plants frequently die off and never make the recovery nor show the stimulation of mustard under similar circumstances. With various intervals between treatment and sowing barley is still killed off at a time of sowing and with a strength of iodine that allows mustard to make its greatest recovery.

6. The various means whereby iodine may be put out of action in the soil are discussed and the suggestion is made that iodine might

possibly have some specific and effective action if means could be found to prevent it from being converted from the elemental state so rapidly as appears usually to be the case.

7. Although no definite partial sterilisation effect has been obtained and though mustard and barley show little or no significant reaction, the possibility is not excluded that other agricultural crops, *e.g.* mangolds and sugar beet, might be benefited by the use of small amounts of iodine in addition to the usual fertilisers.

In conclusion I wish to express my thanks to Dr Bewley, Messrs Sawyer, Cutler, Thornton and Fisher for the help they have freely given in various ways during the progress of the investigations. The active assistance rendered by the Chilean Iodine Committee has already been acknowledged. I am also indebted to Mr Matthews (Shrewsbury) and Messrs H. B. and J. C. Randall (Harpenden and Waltham Cross) for supplying me with the special types of soil needed for experiment.

REFERENCES.

(1) Aso, K. and Suzuki, S. (1904). *Bull. Coll. Agric. Tokyo Imp. Univ.* vi, 159–60.
(2) Bourcet, P. (1899). *Compt. Rend.* cxxix, 768–70.
(3) Cameron, A. T. (1914). *Trans. Roy. Soc. Canada*, iii, Sect. iv, viii, 7–10.
(4) Chatin, Ad. (1850). *Compt. Rend.* xxxi, 280–3.
(5) Cooper, W. F. and Walling, W. A. B. (1915–6). *Ann. Appl. Biol.* ii, 166–82.
(6) Droop-Richmond, H. (1914). *Dairy Chemistry*, 333–5.
(7) Free, E. E. (1917). *John Hopkins Univ. Circ.* new ser. No. 3, 195–8.
(8) Gautier, A. (1899). *Compt. Rend.* cxxviii, 643–9, 1069–75; cxxix, 189–94.
(9) Grandeau, L. (1897). *Traite d'analyse des matières agricoles*, 451–5.
(10) Guichard, P. (1863). *Bull. Soc. Chim.* v, 115, 278.
(11) Heckel, E. (1875). *Compt. Rend.* lxxx, 1170–2.
(12) Lodeman, E. G. (1893). *New York Cornell Sta. Bull.* lx, 255–96.
(13) Mazé, P. (1915). *Compt. Rend.* clx, 211–4.
(14) —— (1919). *Ann. Inst. Pasteur*, xxxiii, No. 3, 139–73.
(15) Moore, P. W. (1916). *Arizona Sta. Rep.* 300.
(16) Scheringa, K. (1911). *Chem. Weekblad*, viii, 11.
(17) Scurti, F. (1906). *Gazetta chimica italiana*, xxxvi, 619–25.
(18) Stanford, E. C. C. (1877). *Chem. News*, xxxv, 172-5.
(19) Suzuki, S. (1903). *Bull. Coll. Agric. Tokyo Imp. Univ.* v, 199–201.
(20) —— *Ibid.* 519–21.
(21) Suzuki, S. and Aso, K. (1903). *Ibid.* iv, 473–8.
(22) Thornton, H. G. (1922). *Ann. App. Biol.* ix, 241–74.
(23) Uchiyama, S. (1905). *Bull. Imp. Cent. Expt. Stat. Japan*, i, No. 1, 35–7.
(24) —— (1907). *Ibid.*, No. 2, 37–79.
(25) Voelcker, J. A. (1900). *Journ. Roy. Agric. Soc.* ser. 3, xi, 566–91.
—— (1901). *Ibid.* lxii, 326–9.
—— (1903). *Ibid.* lxiv, 348–51.
—— (1904). *Ibid.* lxv, 306–14.
(26) Warington, K. (1923). *Ann. Bot.* xxvii, 629–72.
(27) Winterstein, E. (1918). *Hoppe-Seyler's Ztschr. Physiol. Chem.* civ, No. 1, 54–8.

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NOTE ON THE ECONOMIC VALUE OF THE NEUROPTERA, WITH SPECIAL REFERENCE TO THE CONIOPTERYGIDAE

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(With Plate I.)

The order Neuroptera as now restricted is remarkably homogeneous, its uniformity being especially marked in the larval stages. The Sialidae and Raphidiidae are by some authors still included in the true Neuroptera, but it seems better to place them together in a separate order Megaloptera, of which the main distinguishing features are that in the larva the mouthparts are of ordinary biting type and the anus is functional for the voidance of excrement.

In the Neuroptera proper the larva always has mouthparts modified for piercing and sucking, formed by approximation of mandibles and maxillary lobes (? laciniae) to form hollow sucking spears; the anus is closed for the passage of solid excrement and at the end of larval life the anal papilla is used as a spinneret to construct a cocoon with silk secreted by the modified Malpighian tubes. Such larval characters appear to be without exception in Neuroptera (sens. strict.).

All known Neuropterous larvae are carnivorous, and none can be considered as pests[1]. The imagines also commonly live upon a similar diet to the larvae. The order as a whole is beneficial to man.

Up to the present time only five Neuropterous families can be said to merit notice by the economic entomologist, the remaining families, while they may to a certain extent assist in checking injurious insects, yet none are sufficiently prominent to warrant mention here. The five principal families are the Ithonidae, Coniopterygidae, Hemerobiidae, Chrysopidae, and Psychopsidae.

Of these the first and last are confined to the Southern Hemisphere and have been dealt with by Tillyard (25, 26). The larva of *Ithone* is a white, blind, Melolonthoid grub which burrows in sandy soil and preys upon the larvae of Melolonthid beetles (*Odontria* spp. (26)). *Psychopsis* has similar habits to those of *Raphidia*, and as a larva lives under the bark of trees. Its potential value in checking codlin moth larvae and the like is fully discussed by Tillyard (25).

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[1] Vuillet, J. and A. (*L'Agron. Colon.*, Paris, 1914), have suggested that Chrysopids may do more harm than good by preying upon other predacious insects, but this must be considered as an exceptional opinion.

The remaining three families are all represented in the British Isles, and as I have already dealt with the life-histories of several species elsewhere (28), descriptions of these need hardly be repeated here. The Hemerobiidae and Chrysopidae are better known than the Coniopterygidae and therefore it is to the latter family that I would give most attention in the present paper. For further details I must refer to my earlier paper.

Coniopterygidae. The Coniopterygidae have been much neglected by entomologists and are consequently regarded as being rare. Such an opinion is, however, mistaken. Several Coniopterygidae are exceedingly common, at least in England, and where they occur they are generally to be found in numbers. The larvae are small and inconspicuous—hence easily overlooked. Cocoons (Pl. I, figs. 2, 4 and 7) are no doubt frequently passed over as spider's cocoons. The imagines (Pl. I, fig. 5) might, with some excuse, be taken casually for Aleurodoids, or, when on the wing as small white moths such for instance as *Leucoptera*. Actually *Conwentzia* is one of the commonest insects in London parks on oak (*Quercus*) and plane (*Platanus*); *Semidalis* is often abundant in deciduous woods and orchards, while *Coniopteryx* is frequently plentiful in pinewoods in the south of England.

Adult Coniopterygidae differ from all other Neuroptera in their reduced wing venation and in possessing a covering of white waxy powder which is secreted and rubbed over the body of the insect shortly after emergence. They are always of small size, and because of the white powder covering them they resemble Aleurodids superficially. Structurally of course they differ materially from the latter, the most obvious differences found in Coniopterygids being the biting mouthparts, many-jointed moniliform antennae and more complex wing-venation. So far as is known at present no Coniopterygidae have less than fifteen antennal joints, and the wings have several longitudinal veins. The hindwings are reduced in the genus *Conwentzia*.

In the field, the most noticeable difference between Aleurodidae and Coniopterygidae is in the manner of holding the wings at rest. In Coniopterygidae the wings are very steeply roofed over the body (Pl. I, fig. 5), while in Aleurodidae they are much less steeply roofed and are held almost applied to the surface on which the insect is resting. A Coniopterygid when disturbed is often reluctant to fly at once and will either run rapidly about or drop and feign death. Aleurodids take to flight more readily and have quite a different manner of walking. Once the differences in these respects have been noted the most inexperienced observer should have no difficulty in distinguishing between the two insects.

The eggs of Coniopterygidae (Pl. I, fig. 1) are laid singly, and generally at the edges of leaves. The eggs are oval, slightly flattened, with a pointed micropylar apparatus and a reticulate chorion. They average ·4 mm. to ·5 mm. in length. The incubation period varies from six days to three weeks, according to temperature.

The larva is fusiform or top-shaped (Pl. I, fig. 3), smooth or slightly hairy. The head is small, with conspicuous eyes placed laterally, and conical, sucking mouthparts. The antennae are fairly stout and easily visible, two-jointed. The thorax is the broadest part of the body, as a rule, and the legs borne by it are well developed, with the usual Neuropterous unjointed tarsus, two tarsal claws and a small empodium. The abdomen consists of ten segments tapering to the anal extremity. It is often very little longer than the thorax. The length of larva when full fed is from 2 mm. to 3·5 mm.

After two moults, and in from two and a half to four weeks in summer, the larva spins a flat circular cocoon (Pl. I, figs. 2, 4 and 7) of white silk, using its eversible anal papilla as a spinneret. In the case of the first brood cocoons are spun either upon leaves or trunks of trees, but the second brood, which is to hibernate, spins entirely on the trunk. A more or less double cocoon is constructed, but often the inner envelope is imperfect. *Coniopteryx cerata* Hagen, a Ceylon species, spins a most beautiful cocoon with pleats or folds radiating from the centre.

The larvae pupates in a few days in summer and the imago appears about a fortnight after spinning. In the case of the overwintering brood the larva does not pupate, but rests as a prepupa within the cocoon. In spring pupation and emergence occur. The emerging pupa bites a semicircular slit through the cocoon, and the imago escapes from the pupal skin. After the wings have dried, the waxy powder secreted upon the body is scraped off by the hind legs, and, at intervals, is rubbed over and between the wings.

The male imagines of Coniopterygidae are alone attracted to lights at night, the females apparently not being positively phototactic. The imagines feed on similar food to the larvae, but seem to prefer semi-liquid sweet matter, such as honeydew.

The larvae are predaceous upon mites, small Rhynchota, etc. I have never seen a full-grown aphid of medium size attacked. *Conwentzia* *psociformis* larvae, for instance, will prey upon young nymphs of *Vacuna dryophila* Westw., and they feed largely on such in nature, but I have not been able to induce one to take a full grown *Vacuna*.

On the oaks in England the main check upon *Phylloxera punctata* Licht., is frequently *Conwentzia psociformis* Curt. In many parts the damage done to the foliage of oaks by *Phylloxera* is considerable, and some trees seem hardly able to survive the combined attacks of *Tortrix viridana* and of *Phylloxera*. The value of the beneficial work of *Conwentzia* is well testified by the great numbers of cocoons of this insect which may often be seen upon oak trunks during the summer (Pl. I, figs. 1 and 7).

Not only may *Conwentzia* be found preying naturally upon small Rhynchota, but the oak "red spider" (*Paratetranychus quercinus* Berl.) and other mites must be counted among its common hosts. This fact suggested that possibly *Conwentzia* might with benefit be artificially established upon trees infected with common red spider (*Tetranychus telarius* L.). The ideal employment of *Conwentzia* in England would be in such as peach houses, where I feel sure that the Coniopterygid would prove its worth, especially if its parasite *Lygocerus* could be excluded. I am indebted to Professor Lefroy for his efforts to induce certain growers to give this experiment a trial. but none could be persuaded to forego fumigation with nicotine, which of course would prove fatal to *Conwentzia*. I therefore have had to content myself with tests in the open. Various fruit trees infected with mites (*Tetranychus telarius* L., on grape vine; *Bryobia praetiosa* K., on pear and gooseberry; and an unidentified Orobatid on apple) were selected in a neighbourhood where *Conwentzia* had not been previously observed. Cocoons or imagines of the Coniopterygid were placed on the trees. In each case marked success followed. The mites were greatly reduced in numbers and the *Conwentzia* thrived, but every time the parasite *Lygocerus* made its appearance in the second year to the detriment of *Conwentzia*.

The following example may be taken in illustration. A pear tree badly infected with *Bryobia praetiosa* K. was selected, and six newly emerged *C. psociformis* imagines were set free upon the trunk on the 10th May, 1921. From the 15th to the 26th June, 1921, twenty cocoons were counted upon the trunk, and probably more were present on leaves and twigs. By the 8th July more cocoons had appeared. On the 20th July over one hundred cocoons were counted on the pear trunk, thirty on another contiguous pear, and six on an apple. On the latter tree the most probable food for *Conwentzia* observed was young migrating *Eriosoma lanigerum* Hausm., and a few Psocids. About 30 per cent. of the cocoons of the 20th July did not yield imagines in 1921 but hibernated along with the true second brood. There were quite possibly many second brood cocoons among these and one could not say definitely under such conditions of observation. Between the 27th July and the 4th August imagines escaped from the greater part of 70 per cent. of the cocoons. These empty cocoons were then removed in order to make a fresh count of the true second brood. The second and last brood of the year were spinning up in large numbers on the 14th August. Larvae continued spinning in decreasing numbers into September. It was now seen that six or seven neighbouring pear trees also bore numbers of *Conwentzia* cocoons. An exact count could not be made, but it would doubtless have exceeded one thousand cocoons.

In September and October the cocoons were observed to be attacked by larvae of *Chrysopa tenella* Schn., which pierced the cocoons with their jaws and sucked out the juices of the contained larvae. In July 1922 a single parasite *Lygocerus* was noticed on the bark of the pear. The second brood of *Conwentzia* was much smaller than that of 1921. It was found to be heavily parasitised by *Lygocerus*, and a further reason for the reduced numbers was the fact that no specimens of its previous food, *Bryobia praetiosa* K., were now to be found. It seems quite clear that *Conwentzia* was largely responsible for the disappearance of the mite.

From this experiment alone, and similar cases could be quoted, it seems clearly possible to employ *Conwentzia* as a check upon various pests, especially against such as red spider. Given favourable conditions and absence of parasites I consider that there are unusual possibilities with *Conwentzia psociformis*. Such conditions would probably be obtained in England under glass. It is decidedly unfortunate that this has not been put to the test and that there is no positive evidence to offer. However, the suggestion is put forward and it is hoped that some future worker on this family will make the experiment, which, if inferences can be drawn from outdoor results, should prove highly successful. Probably *Conwentzia* would also prove efficient in other countries where the genus does not exist if it can be introduced there without its parasites.

The following method of establishment might be tried. To avoid the parasite *Lygocerus* collect larvae of *Conwentzia psociformis* when they swarm on the oak trunks in August[1]. Transfer these to a box with corrugated paper, or the like, upon which they will readily spin. Thus they can easily be handled and packed. *Lygocerus* attacks the cocoon and bites a hole in it to deposit an egg, therefore collection of larvae before spinning should exclude this parasite. There are other parasites to be expected, however. Should the *Conwentzia* larvae not be taken when they are about to spin, only such cocoons should be collected as appear unperforated. These are more likely to have been untouched by *Lygocerus*, but there is still a risk.

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[1] July or September in some years.

Chilling, as in winter, appears to be necessary before the second brood larvae (prepupae) will pupate, so that for shipping no special precautions seem to be indicated to prevent emergence *en route*. This, however, requires to be further tested.

One dozen cocoons attached to the bark of a tree should be sufficient to start a colony. The emerging insects do not fly readily and will, if food is present, usually remain on the tree to which transferred. Thus pairing and egg laying should certainly follow. It is far better, however, to place newly emerged imagines and not cocoons on the trees if there is the slightest possibility of any cocoons being parasitised. This is bound to be the case with wild cocoons.

In introducing Coniopterygidae to a foreign country parasites must be rigidly guarded against. Up to the present two Chalcid parasites of *Conwentzia psociformis* have been found. The life histories of these have not yet been fully worked out, but both have been bred from cocoons. *Lygocerus* sp. tears a hole in the cocoon of *Conwentzia psociformis* in late summer or autumn and then lays one egg within. The parasite larva (Pl. I, fig. 8) is full fed before winter and it pupates in early summer of next year. In July the adult parasite appears and the cycle probably recommences. Thus it would seem that *Lygocerus* is single brooded, but I have some observations of a possible double brood.

A little experience will enable one to detect the full fed larva of *Lygocerus* within a *Conwentzia* cocoon in winter. It is only necessary to remove the outer envelope of the cocoon when the orange-pink parasite larva will be seen within. Usually the small hole torn in the envelope by the parent *Lygocerus* is distinctly visible from outside, but parasitised cocoons have been found in which a hole had not previously been detected. The best way to avoid *Lygocerus* is to collect full-fed larvae of *Conwentzia* just before spinning and allow them to spin in captivity.

A second parasite, *Ceraphron* sp., has emerged from cocoons of *Conwentzia psociformis* during July and August. Two females came from each of eleven cocoons. I know very little of the life history of this species and therefore can advise no preventive measures.

A great drawback to the introduction of Coniopterygids into greenhouses is that growers may confuse them with Aleurodids. In the case of *Conwentzia* this confusion need hardly occur as this insect is much larger than *Aleurodes vaporariorum* and once the two insects have been compared in life a person with no scientific training should at once be able to distinguish between them. It has already been pointed out that the manner of holding the wings at rest, antennae, etc., are totally different.

In giving attention to the Coniopterygidae it is desired to point out that there appear to be potentialities in this Neuropterous family which have been overlooked and a trial of the foregoing suggestions seems well worth while. The writer is not in a position to continue this work at present and the evidence so far obtained is insufficient, but all results, as far as they have gone, have been highly satisfactory and there seem to be considerable possibilities. The Coniopterygidae, but more especially those species of the genus *Conwentzia*, appear to lend themselves well for experiment. They are semi-gregarious and fly little, thus they tend to become abundant locally and do not leave one plant until scarcity of food compels. Each female lays a fair number of eggs, probably about two hundred in nature, as it continues to live and feed for a month or so. The cocoons are conspicuous, being spun in the open, and they can thus be easily collected and distributed. The one necessary precaution is against the introduction of parasites with the cocoons. Apparently the larva itself is not parasitised while free, but this assertion must not be taken as necessarily without exception.

In America notices of *Conwentzia hagani* Bks., preying upon red spider (*Tetranychus telarius* L.) and other mites damaging fruit trees (citrus and cherry) have appeared by Quayle (19, 20) and Houser (12). This species seems to be very similar in habits to our *Conwentzia psociformis* Curt.

Hemerobiidae. The Hemerobiidae as a whole seem to prefer trees to low-growing plants. They are therefore found mainly in woods and forests. In the south of England the commonest species in orchards and gardens is *Boriomyia subnebulosa* Steph. This insect is often found as a larva on low herbs.

The egg is attached by its side to the undersides of leaves of plants, also concealed in crevices, etc. It is elongate oval in shape, smooth, but with the chorion finely pitted, as a rule. There is a micropylar knob more or less distinct. In length eggs vary from ·4 mm. to 1 mm. Colour at first is generally white or yellowish-white. The incubation period in summer varies from six days to two weeks.

The larva in the first instar alone has a trumpet-shaped empodium between the tarsal claws. In later life this is reduced to a small and inconspicuous pad. A Hemerobiid larva can thus be distinguished from a Chrysopid by the absence of the trumpet form of empodium in later life. Other characters of the Hemerobiidae are the shorter, stouter jaws, and the long, fusiform and almost nude body. The body of the Hemerobiid larva never bears setigerous tubercles and never in consequence carries rubbish upon the back. Larvae when full-fed vary from 4 mm. to 1 cm. or more in length. The active larval period in summer averages ten to fourteen days.

The cocoon is spun among leaves or in a crevice of bark, etc. It is oval in shape and of fairly open mesh so that the insect can usually be seen within.

The life cycle varies considerably. There may be one or many generations in a year. Winter is passed in all stages, according to species.

The British species vary too greatly in their feeding habits to enable one to assign any species to particular trees. The following with their preferences are given as a guide, in case it should be desirable at any future date to employ Hemerobiids against special pests.

CONIFERS. *Sympherobius inconspicuus* McL., *Hemerobius nitidulus* Fab., *H. orotypus* Wallengr., *H. stigma* Steph., *H. pini* Steph., *H. limbatellus* Zett., *H. atrifrons* McL., *H. mortoni* McL., *Boriomyia rava* Withyc., *B. concinna* Steph., *B. quadrifasciata* Reut.

DECIDUOUS TREES. *Sympherobius pygmaeus* Ramb. (oak), *S. elegans* Steph. (beech), *Hemerobius micans* Oliv. (beech), *H. humuli* L. (oak, beech, hazel, etc.), *H. lutescens* Fab. (oak, beech, hazel, etc.), *Boriomyia subnebulosa* Steph. (various), *B. nervosa* Fab. (birch, etc.), *Micromus paganus* L. (low herbage).

Of this list the most common species may be taken as *H. stigma* Steph., in pinewoods, and *B. subnebulosa* Steph., everywhere. The latter species is very common in gardens near London and may be easily obtained at light. The larva often feeds on *Aphis rumicis* L., on broad beans and is also found commonly upon apple trees, but it is not at all particular as to diet.

In America we find *Sympherobius angustus* Bks., recorded by Essig (3) as feeding upon the citrus mealy bug (*Pseudococcus citri*) in California. *Hemerobius californicus* is noted by Quayle (20) as preying upon red spider. Other records are given in the bibliography.

Chrysopidae. The Chrysopidae or Green Lacewings are well known, and their value in checking aphids in gardens and orchards need not be expatiated upon.

The eggs, which are usually a little less than 1 mm. long, are of long oval shape, smooth, and with a saucer-shaped micropylar apparatus. The colour is generally greenish. The antimicropylar pole is attached by a hair-like stalk to a leaf or twig. The stalk varies in length from about 2 mm. to 9 mm. Eggs are laid singly, in spaced clusters, or in dense clusters with stalks united into a bundle. The number of eggs laid by a single female varies from one to several hundred. (I have obtained four hundred eggs from a female of *Chrysopa phyllochroma.*) The period of incubation averages just over a week in summer, but varies according to temperature.

The larva is stouter in build than most Hemerobiidae. It is also generally much more hairy, and in most species the larvae have tubercles from which arise tufts or brushes of stiff hairs. The empodium between the tarsal claws is trumpet-shaped throughout life. The larval period averages about three weeks in England.

For pupation a closely woven, parchment-like oval cocoon of silk is spun among leaves or under bark, etc. Winter is generally passed as a prepupa, but sometimes as a larva or imago.

To conifers *Chrysopa dorsalis* Burm., and *Nothochrysa capitata* Fab., are especially attached, but *C. vulgaris* Schn., *C. flavifrons* Br., *C. prasina* Burm., *C. ventralis* Curt., and *C. perla* L., are also commonly to be met with in pinewoods. *C. prasina*, in particular, is most often found feeding upon *Chermes* in young pine plantations.

On deciduous trees one may find *Chrysopa flava* Scop. (oak, beech, hazel, sycamore), *C. vittata* Wesm. (oak, beech, hazel), *C. vulgaris* Schn. (various trees and shrubs), *Chrysopa tenella* Schn. (various trees and shrubs), *C. alba* L. (shady woods), *C. flavifrons* Br. (various trees and shrubs), *C. prasina* Burm. (oak and beech), *C. ventralis* Curt. (oak and beech), *C. septempunctata* Wesm. (gardens and orchards), *C. perla* L. (low woodland and hedges), *C. phyllochroma* Wesm. (bean-fields).

As most important of these may be selected *Chrysopa prasina*, *C. flava*, *C. vulgaris*, *C. tenella*, *C. septempunctata* and *C. phyllochroma*.

C. prasina as a larva is undoubtedly a valuable check upon *Chermes* in young pine plantations. It is one of the very few predaceous insects which will attack this aphid and its activities continue throughout the winter. The larvae may be found, concealed by the dense packet of aphid wool and skins upon their backs, crawling over the trunks of the trees during any warm spell of the winter months. In summer larvae can be beaten from the branches. Similar remarks will apply to *C. ventralis* and *C. flavifrons* but these two species are usually less common. The larvae of all three species will live for weeks without food if kept at a temperature between 32° F., and 40° F.

C. flava is a species which seems to prefer larger Aphids, Psyllids and Jassids in its larval state and I have sometimes failed to keep larvae at all on aphids such as *Aphis rumicis* L., and *Aphis* (*Brevicoryne*) *brassicae* L. Although extremely voracious, attacking even spiders and beetles in nature, it is curious that at times the larvae should prove so delicate in captivity. The species seems to be especially attached to oaks.

C. vulgaris is not common in England as a rule. The larva is almost omnivorous as far as small insects and mites are concerned. In the continent of Europe and throughout all the Mediterranean region it is abundant, judging by the frequency with which it appears in collections. From France it is commonly reported (2, 7, 8, 9) upon grape vines where it preys upon larvae of *Polychrosis botrana*, *Phylloxera* and red spider. Imagines are frequently caught in sugar traps set for *Polychrosis*. *Chrysopa vulgaris* hibernates as an imago.

The larvae of *Chrysopa tenella* and *C. septempunctata* may be given mention as feeding upon *Eriosoma lanigerum* Hausm., on apple. So few natural enemies of apple woolly aphis are known that this is worth noting. In tastes both species are not at all fastidious and many other orchard and garden insects are also preyed upon. *C. septempunctata*, for instance, is a common check upon *Aphis rumicis* L., on beans. Both *C. tenella* and *C. septempunctata* hibernate as prepupae within the cocoon.

Chrysopa phyllochroma, Mr B. S. Harwood tells me, is often found by him in bean-fields, where its probable food is *Aphis rumicis*. Mr Harwood sent me one female of this species from which over four hundred eggs were obtained. Both imago and larvae fed readily and thrived upon *Aphis rumicis*. The larva can be described as somewhat like a slightly elongate *C. tenella* larva in general appearance, the colour being grey, but there are several differences in detail which will be described in a subsequent paper.

In America the principal papers dealing with Chrysopids from an economic standpoint are those of Wildermuth (27) and Smith (21, 22). The former gives the life history of *Chrysopa californica* Coq., and includes a list of host insects comprising mites, Jassids, Psyllids, Aphids and Coccids. Smith (22) has given a very complete account of the life histories of several American Chrysopids and to his excellent paper reference should be made.

In Australia Chrysopids are mentioned as preying upon Aphids (11) and Coccids (10).

In this paper it has been my endeavour to give only such details as are likely to be of value in the selection and naturalisation of Neuroptera for economic purposes. Details of the insect's length of life, fecundity, food, etc., are omitted not because they are unimportant but because there is such great variation that without much more complete evidence, under greater variety of conditions, such details might convey erroneous impressions. Personally I have only been able to observe the British species near London, and under consequent very limited environmental conditions. Where preference in food is assigned to certain species this is always from observations in the field and not in captivity.

As regards the amount of food taken, a brief mention of this was given in my recent biological paper (28). In adopting a standard size aphid as food throughout, and in compelling even the first instar larvae to feed upon such aphids, a much smaller total of aphids eaten during life was obtained than that of most previous observers. Of course in nature a newly hatched larva would for preference attack aphids smaller than itself at first, but in my experiments this preference was repressed. If the larva is given smaller aphids at first it is natural to expect that a larger figure for the total number of aphids eaten will be obtained. For further details of amount of food taken by Neuropterous larvae see Quayle (19) for Coniopterygidae, Quayle (20) for Hemerobiidae, Wildermuth (27) for Chrysopidae.

The parasites attacking Neuroptera have been also omitted here except in Coniopterygidae. There is still much to be learned concerning these and a preliminary list of British parasites has already been given (28). Others have since been found. In Chrysopidae Hymenopterous parasites attack eggs, larvae, and prepupae. The imago alone can be considered free, and this only in the absence of evidence to the contrary.

The present paper is merely offered to fill certain gaps in the literature and to suggest promising lines for future investigation. It is hoped that the facts here reviewed may be of assistance to the economic entomologist.

SUMMARY.

1. The Neuroptera as a whole are distinctly beneficial to man. Three families of economic importance are represented in Britain; the Coniopterygidae, Hemerobiidae, and Chrysopidae.

2. The Coniopterygidae are far more common in England than is

generally supposed. In general facies they resemble Aleurodidae, but can easily be distinguished.

3. The eggs are laid generally at the edges of leaves. Larvae are top-shaped, with small head and mouthparts. They are predacious. After two moults a flat, circular cocoon is spun.

4. Larvae of *Conwentzia* are very beneficial on oaks in England, where they prey upon small Rhynchota, such as *Phylloxera*, and mites.

5. They would seem to lend themselves particularly well to employment economically and might be introduced to peach houses in England.

6. Six imagines of *Conwentzia* released upon a pear tree infected with *Bryobia praetiosa* K., resulted in roughly one thousand individual cocoons at the end of the season 1921.

7. *Conwentzia* could probably be introduced successfully into other countries where the genus does not exist. Parasites must be rigidly guarded against.

8. The Hemerobiidae are more often found in woods than elsewhere in Britain. Some shew preference to conifers, others to deciduous trees. *Boriomyia subnebulosa* Steph. is common in orchards and gardens near London.

9. The larvae of Chrysopidae differ from Hemerobiidae by possessing throughout life a trumpet-shaped empodium, and generally setigerous tubercles.

10. As in Hemerobiidae, larvae, according to species, shew preferences to different trees.

11. *Chrysopa prasina* Burm. is a valuable check upon *Chermes*, on conifers; *C. tenella* Schn., and *C. septempunctata* Wesm., on *Eriosoma lanigerum* Hausm., of apple; while *C. phyllochroma* Wesm., probably does valuable work in bean-fields in some parts.

BIBLIOGRAPHY.

(1) ARROW, G. J. (1917). The Life-history of *Conwentzia psociformis* Curt. *Ent. Mo. Mag.* LIII, 254–7.

(2) CONSTANT, A. (1881). "Hemerobius" larva feeding on larvae of *Pempelia euphorbiella. Bull. Soc. Entom. France,* VI, 1, pp. xxi–xxiii.

(3) ESSIG, E. O. (1910). The Natural Enemies of the Citrus Mealy Bug. I. *Pomona Coll. Journ. Ent.* Claremont, Cal., II, 143–6.

(4) —— (1911). Natural Enemies of the Citrus Plant Lice. *Pomona Coll. Journ. Ent. Cal.* III, 604–16.

(5) —— (1914). The Mealy Bugs of California. *Monthly Bull. Sta. Commiss. Hortic.* Sacramento, Cal. III, No. 3, 97–143.

(6) EWING, H. E. (1914). The Common Red Spider or Spider Mite. *Agric. Exp. Sta. Oregon, Bull.* No. 121, 1–95.

(7) Feytaud, J. (1913). *Cochylis* et *Eudemis*; procédés de capture des papillons. *Bull. Soc. d'Étude et de Vulgar. de la Zool. Agric. Bordeaux*, No. 4, 97–104.

(8) —— (1913). Les Hémérobes ou Chrysopes. *Bull. Soc. d'Etude et de Vulgar. de la Zool. Agric.* v, 138–48.

(9) —— (1913). Recherches sur la *Cochylis* et l'*Eudemis* dans le Bordelais en 1912. *Annales des Epiphytes*, pp. 256–7.

(10) French, C. (1912). Mealy Bugs destroyed by Lacewings. *Journ. Dept. Agric. Victoria*, x, 485.

(11) Froggat, W. W. (1904). Experimental Work with the Peach Aphis. *Agric. Gaz. N.S.W.*, xv, Pt 7, 603–12. See also *Proc. Linn. Soc. N.S.W.*, 1904.

(12) Houser (1914). *Conwentzia hageni* Bks., Life-history, etc. *Ann. Ent. Soc. Amer.* vii, 73–6.

(13) Lacroix, J. L. (1921). Études sur les Chrysopides. Premier Mémoire. *Ann. Soc. Linn. Lyon*, lxviii, 51–104.

(14) McGregor, E. A. and McDunnough, F. L. (1917). The Red Spider on Cotton. *U.S. Agric. Dept., Bull.* 416, 1–72.

(15) Moffat, J. A. (1900). Parasites in the Eggs of *Chrysopa*. *Rep. Ent. Soc. Ontario*, 51–2.

(16) Moznette, G. F. (1915). *Second Biennial Crop Pest and Horticultural Report*, 1913–14. Oregon Agric. Exp. Sta. Corvallis, 288 pp. *Hemerobius pacificus* Bks., pp. 181–3.

(17) —— (1915). Notes on the Brown Lacewing (*Hemerobius pacificus* Bks.). *Journ. Econ. Ent.* viii, 350–54.

(18) Perkins, R. C. L. (1905). Leaf-hoppers and their Natural Enemies. *Hawaian Sugar Planters' Assn. Exp. Sta. Ent. Div. Bull.* i, 174–7.

(19) Quayle, H. J. (1912). Red Spiders and Mites of Citrus Trees. *California Univ. Agric. Exp. Sta., Bull.* 234, 479–530. *Conwentzia hageni* Bks., Life-history, pp. 506–9.

(20) —— (1913). Some Natural Enemies of Spiders and Mites. *Journ. Econ. Ent.* vi, No. 1, 85–8.

(21) Smith, R. C. (1921). A Study of the Biology of the Chrysopidae. *Ann. Ent. Soc. Amer.* xiv, 27–35.

(22) —— (1922). The Biology of the Chrysopidae. *Cornell Agric. Exp. Sta., Memoir* No. 58, 1287–1372.

(23) Terry, F. W. (1905). Leaf-hoppers and their Natural Enemies. *Hawaian Sugar Planters' Assn. Exp. Sta., Bull.* No. 1, Pt v, 174–7.

(24) —— (1908). Notes on the Life-history of an Endemic Hemerobiid (*Nesomicromus vagus* Perk.). *Proc. Hawaian Ent. Soc. Honolulu*, i, 174–5.

(25) Tillyard, R. J. (1918). The Life-history of *Psychopsis elegans* Guer. *Proc. Linn. Soc. N.S.W.* xliii, Pt 4, 787–818

(26) —— (1922). The Life-history of the Australian Moth Lacewing, *Ithone fusca* Newm. *Bull. Ent. Res.* xiii, Pt 2, 205–23.

(27) Wildermuth, V. L. (1916). California Green Lacewing Fly. *Journ. Agric. Res. Washington*, vi, No. 14, 515–25.

(28) Withycombe, C. L. (1922). Notes on the Biology of Some British Neuroptera. *Trans. Ent. Soc. London*, pp. 501–94.

(29) Zehntner, L. (1900). De Plantenluizen van het Zuikerriet op Java. X. *Arch. Java suikerindus*, Afl. xx, 1–32.

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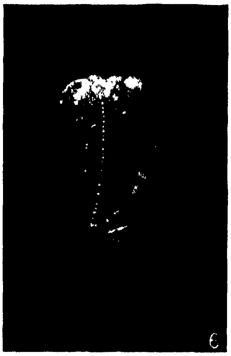

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WITHYCOMBE.—Note on the Economic Value of the Neuroptera, with Special Reference to the Coniopterygidae.

EXPLANATION OF PLATE I

Conwentzia psociformis Curtis

Fig. 1. Egg of *Conwentzia psociformis* Curt., on edge of oak leaf, underside. (× 12.)
Fig. 2. Parasitised cocoon of *Conwentzia psociformis* Curt., on oak leaf. (× 1.)
Fig. 3. Full fed larva of *Conwentzia psociformis* Curt. (× 4.)
Fig. 4. Cocoons of *Conwentzia psociformis* Curt., on bark of oak tree. A portion of Fig. 7 to a larger scale.
Fig. 5. *Conwentzia psociformis* Curt., imago. (× 4.)
Fig. 6. Pupa of *Conwentzia psociformis* Curt., removed from cocoon. (× 12 approx.)
Fig. 7. Cocoons of *Conwentzia psociformis* Curt., on trunk of an oak tree in Epping Forest.
Fig. 8. Parasite (*Lygocerus* sp.) larva full fed. Cocoon opened in winter. (× 4.)

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A NOTE ON CUTWORM BAIT EXPERIMENTS

By WILLIAM C. COOK.

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THERE appeared in this *Journal* for July, 1923, an article by Fryer and Stenton(1) in which experimental data were presented showing the development of four laboratory methods of determining the attractiveness of cutworm baits. The writer of this note has been occupied with cutworm studies for several years, and, while working at the Minnesota Agricultural Experiment Station, developed a method for such experiments quite similar to the unsuccessful technique of Fryer and Stenton in their "Series I," but which has proved uniformly successful, and may be of interest to readers of this *Journal*.

The method followed, which was undoubtedly suggested by some experiments of Dr R. N. Chapman(2) on the selection of food by *Tribolium confusum*, was to liberate a given number of larvae in the centre of a circle, around whose circumference were placed alternating piles of two unpoisoned baits to be tested. In practice, sixteen piles of the baits, spaced evenly around the periphery of a circle two feet in diameter, were found to be a sufficient number to give good results. The cage was darkened and record was made of the number of larvae feeding on each bait after a half hour or so.

It was found necessary to place each pile of bait on a paper, and to place a pile of loose sand in the centre of the circle to eliminate such larvae (usually nearly half of the total number) as were seeking shelter and not food.

This method has proved entirely reliable and repetitions of a given test with several different lots of larvae of the same species and age have always given very nearly identical results.

REFERENCES.

(1) FRYER, J. C. F., and STENTON, R. (1923). Notes on the control of "cutworms" by poisoned bait. *Ann. Appl. Biol.* x, No. 2, pp. 241–52.

(2) CHAPMAN, R. N. (1918). The confused flour beetle (*Tribolium confusum* Duval). In *17th Rept. State Entomologist of Minnesota*, 1918, pp. 73–93 (Food selection expts. on p. 79).

REVIEWS

Botanical Pen-Portraits. By J. W. MOLL and Dr H. H. JANSSONIUS. Martinus Nijhoff. The Hague. 1923. 30 Gld.

This is an astonishing volume and something quite new in botanical literature. It is a minute description of the macroscopic and microscopic anatomical characters of one hundred or so common drugs and is a large well packed volume. Anatomical descriptions are, as a rule, dull enough reading, but these compel one's interest: the fullness of detail is a thing never before attempted and one is left gasping with the feeling that this knowledge is complete and final. In a long preface which is really a very fascinating essay written apparently by Dr Moll, the general history of descriptive method in pharmaceutical botany is briefly sketched leading up to an account of the micrographic method of the present volume. In reading this delightful essay it is interesting to find one's mind constantly reverting to de Candolle's 'Phytographie' and one is astonished to find how far sighted and modern many of his views were. In very largely abandoning illustration by line drawings and in elaborating their method of pen-portraiture which is in essence minutely accurate, quantitative, and perfectly systematised three-dimensional description, the authors have made a very important advance in pharmaceutical diagnostics.

Following the preface are some twenty pages of 'Guiding Schemes' which are minutely analysed key-schedules for guidance in writing pen-portraits of any particular structure. These schemes are very completely worked out and should prove of very considerable service in systematising anatomical description in general phanerogamic botany quite apart from their use in pharmaceutical studies. In any system of pen-portraiture rigid schedules such as these are obviously essential. The authors' schemes are the fruits of long years of experience and a rare capacity for taking pains and as guiding schedules seem almost perfect of their kind.

The book itself is a series of examples of the use of the guiding schemes applied to various common drugs. In the arrangement of the pen-portraits the authors have made a very sensible and long-overdue departure from common pharmaceutical practice. In the ordinary Pharmacopoeias the name of the plant from which the drug is obtained precedes the morphological structure in which it is found, e.g., *Coriandri Fructus*, *Hydrastis Rhizoma* and so forth, and in this way the drugs are scattered over the pages in a totally irrational and very inconvenient way. In the present work the much more logical and useful sequence is adopted of transposing the binomial names, grouping together the drugs from homologous plant organs and within each group arranging the plant names alphabetically. In this way for example *Lignum Guaiaci* is followed by *Lignum Quassiae* and not by some rhizome or leaf drug from a totally unrelated plant whose name chances to begin with the letter G.

The pen-portraits are in themselves of considerable interest and shew what can be accomplished by the micrographic method when backed by almost superhuman patience and industry. One of the most striking and valuable aspects of the portraits is the way in which quantitative methods have been used. In 1919 MacLeod published a book on *Quantitative Method in Biology* which has been far too much overlooked in anatomical, pharmaceutical and other lines of research in which the application of many of the quantitative methods discussed by MacLeod are vitally applicable. Moll and Janssonius apparently unacquainted with MacLeod's work have yet applied quantitative method in many of their micrographic studies and their valuable results take one an appreciable step towards the ideal of the reduction of anatomical diagnoses to a numerical formula.

The only book in any way comparable with the present one which the reviewer has actually used with any degree of laboratory intimacy is the Oxford translation by Boodle and Fritsch of Solereder's great work on the *Systematic Anatomy of*

Dicotyledons. In this, very different descriptive methods are followed and it is only by comparison with such a treatise that one realises the advance in accuracy and precision and completeness of description which Moll and Janssonius have attained in their work.

In addition to the contents already mentioned the volume contains a complete list of reagents used in the pharmaceutical examination of drugs, a very full and useful bibliography, a glossary and a good index. The book is one which will interest all anatomists and students of pharmacy and which will be of the utmost service to research workers attempting anatomical descriptive work. Moll and Janssonius have in fact set a new and much higher standard in botanical description. The volume is written in English and beautifully produced, but its pages contain too many misprints.

WM. B. BRIERLEY.

Diseases of Crop Plants in the Lesser Antilles. By WILLIAM NOWELL, D.I.C. (West Indian Committee, 14 Trinity Square, London, 1923. 12*s*. 6*d*.)

The attention of English speaking peoples was first called to diseases of tropical crops when the coffee industry in Ceylon was destroyed by *Hemeleia vastatrix*. Even a disaster of this magnitude failed to bring home to the minds of tropical agriculturists the vital importance of plant diseases as a limiting factor in crop production and the progress of knowledge was very slow. In the succeeding years a few special treatises were published such as Watt's *Pests and Blights of the Tea Plant* and Wakker and Went's *De ziekten van het Suikerriet* both in 1898 and from time to time smaller brochures dealing with specific troubles. Only in 1911 appeared the first general compendium, *Maladies des Plantes Cultivées dans les Pays Chauds*, by Delacroix and Maublanc and this was followed in 1913 by *Diseases of Tropical Plants* by M. T. Cook the American plant pathologist. Since then no comprehensive and general account of tropical diseases has been published but special treatises such as those of Petch on *Diseases of the Tea Bush* and *Diseases and Pests of the Rubber Tree* have appeared. In 1918 Butler set a new standard by the issue of his *Fungi and Disease in Plants*. This is a masterly survey of diseases of field and plantation crops in India and incidentally a very remarkable book consisting as it does very largely of the personal work of the author and his colleagues. The fine example set by Butler has been followed by Nowell of the West Indian Department of Agriculture who has just published a treatise on *The Diseases of Crop Plants in the Lesser Antilles*.

In many ways the latter volume invites comparison with Butler's volume. Both are written by research men having very special and intimate knowledge of the particular crops in the areas with which they deal and both write with a ripe experience of their problems. Both books are based essentially on the same plan—a first part dealing with the nature of fungi, their food requirements and life histories, the causation of disease and the principles and methods of disease control; followed by a second part containing an almost monographic treatment of diseases of special crops, many of which are common to both areas.

The Lesser Antilles are of course microscopic compared with India and it is almost inevitable that this difference should be reflected in the two volumes. Nowell's effort, admirable though it be, is perhaps a little parochial compared with the wider vision and grasp of Butler's volume. Still that is no detriment in a volume written primarily for parochial purposes and that one should compare it with Butler's work is in itself to give it high praise.

Concerning volumes such as these one may hazard a remark with which perhaps there may be much disagreement. The distinctive part of such works is the consideration of the diseases of the special crops in the particular areas. The introductory parts dealing with fungi and disease in general are not obligatorily related to the special parts and in these two volumes might almost be interchangeable or have been published entirely separately. In the early volume by Delacroix and Maublanc the general part occupies about one-half the work; in that by Cook about one-quarter, in Butler's volume about the same and in Nowell's about one-third. The two latter volumes will it is hoped be followed by others from mycologists in various Overseas

Dominions but one may perhaps suggest that unless the authors have some definite contribution to make to the general theory of plant disease or some new interpretation or improved arrangement which demands expression, there is no obligation on them to occupy a large part of their volumes with general introductory matter. For this, they can refer to Butler, Nowell or even general text-books and confine themselves to their own special subject which is their real contribution.

One of the most welcome and promising signs in plant pathology to-day is the marked tendency for the plant pathologist to get away from the domination of academic mycology, a fetter which tradition has placed upon him and which is in no way more vital to his work than physiology and agriculture including soil physics and chemistry. Nowell's attitude is very sane. He says: "As a result of personal experience of which the whole tendency has been to compel a mycologist to become an agriculturist the writer is convinced of the need for a broad treatment of his subject. The mycologist is most concerned with the fungus, the pathologist with the disease, and the agriculturist with the crop, but a combined view of the interests of all three is necessary for the production of an economic plant pathology."

It is impossible in this review to discuss critically the mass of information in Nowell's volume. Many of the problems considered are still *sub judice* and the author takes a wise and discreet middle course stating in a very fair way the conflicting opinions and evidence.

The West Indian Department of Agriculture has reason to be proud of its mycological staff for the work under review is to a very large extent built up on the author's own observations supplemented by those of Howard, Lewton Brain, Stockdale and South, predecessors in his post. The work is illustrated by text-figures and many beautiful photographs but there are one or two of the latter which might with advantage be brought up to the general standard. Figure 109 is not good and the poor reproduction of Engler and Prantl's very inadequate Pflanzenfamilien picture of Corn Smut in figure 99 does very scant justice to the appearance of this disease in the field. A good photograph from nature would be a decided improvement. Text-figure 1, reproduced again in text-figure 124 as "*Thielaviopsis* in Cells of Sugar-Cane" is distinctly crude. The author apologises for his bibliography and it really is a pity that he did not ask a friendly colleague to complete it for him for it is a serious lapse in a very fine piece of work.

The volume is published on behalf of the Imperial Department of Agriculture by the West India Committee and one can only congratulate the authorities concerned on the wisdom of their action and on their good fortune in having Nowell to prepare the text. It only remains to add that the volume contains a justly appreciative foreword by Professor Farmer, that it is very sensibly produced (which cannot be said of certain recent botanical volumes!) is commendably free from misprints and has the very great virtue of being issued at 12*s*. 6*d*. It is a volume that will be invaluable to all students and practitioners of tropical agriculture in West Indian and similar areas and one that all technical plant-pathologists will find a very useful addition to their library.

WM. B. BRIERLEY.

The Principles of Insect Control. By ROBERT A. WARDLE, M.Sc. and PHILIP BUCKLE, M.Sc. (Manchester University Press: Longmans Green.) 20*s*. net.

The literature devoted to Economic Entomology during the last twenty or thirty years is enormous. It is scattered throughout Agricultural, Medical and other publications; it is often buried in obscure journals and Experiment Station bulletins, and much is therefore inaccessible to many workers of this branch of science and hardly available at all to the practical man. The labour involved in collecting so much material and in giving a just review of it would have daunted most men, yet this has been adequately accomplished by the authors. Moreover this modern entomological warfare is no longer a question of small skirmishes and rule of thumb methods, of occasional battles and retirement to winter quarters. So intimate are the relationships of insect life to the well-being, both physical and economic, of mankind that it demands the unceasing vigilance of a considerable army of highly trained men in every civilised country, who apply almost every type of scientific knowledge to the prosecution of their campaign. Biology, chemistry, physics, meteorology, engineering, have contributed and each year do so on an increasing scale to our knowledge of the best ways and means of combating insect attack. This book may well be regarded as a War-book; it is an attempt to coordinate within a limited scope all the intricate and complicated strategy so far elaborated for this purpose.

It is impossible in a short review to survey even in the briefest detail the mass of material gathered together here. The authors divide their subject into four parts dealing with the methods employed in Biological, Chemical, Mechanical and Legislative Control. The first three of these are further sub-divided into theses which deal in brief detail with the fundamental principles involved and with the practical means of applying them. It is of course obvious that such a treatment is in some measure artificial. For example a good proportion of the matter dealing with mechanical control by 'baits and traps' and in 'crop storage' is largely chemical in essentials, and 'cultural methods' are not necessarily merely mechanical. Such treatment however despite its somewhat illogical nature does enable one to pigeon-hole a complicated mass of fact. It is also obvious that a book of this type must be a compilation and an expert would be needed to deal completely with each of the chapters. Yet these are not disjointed digests of work done in many fields, but are linked together and welded, where possible, into a complete story, with a word or two of shrewd criticism sprinkled here and there.

Perhaps the one unsatisfactory chapter in the book to the reviewer is that dealing with Parasites and Predators. To the non-specialist reader who depends upon digests of the literature of this type for his general information, it would appear too condensed to be clear while for the expert it is probably too scanty for purposes of reference.

The chapters dealing with Insecticides are quite admirable. Criticism can only be levelled at minor points. Shafer's fundamental work might have been described in greater detail, and it is to be hoped that those readers who carefully peruse the excellent bibliography attached will turn up the original account of his researches. But on the whole the present state of our knowledge about the fundamentals of insecticidal action are well reviewed. It is admitted by all that it is still very incomplete and there is probably much set down here that will have to be revised later in detail. For example our information as to how insecticides penetrate the chitin, is still lamentably small, and it is probable that gases and vapours have a different mode of action from liquids and solids. The authors indicate one important essential, that solids must fulfil, if they are to be used as insecticides—they must be finely dispersed; this not only because powders tend to settle more rapidly and to clog nozzles but because toxic intensity would appear to depend directly upon fineness of division.

On page 74 there is an error in the equation dealing with 'the poison exponent' of insecticides, while on page 125 in the account given of Facer's method of analysing tank fluids, a very precise chemist would probably prefer the term arsenious to arsenic oxide. These are small matters but the statement occurring on page 197, dealing with the toxicity of chloropicrin to human beings might have grave consequences. It needs the clearest recognition that chloropicrin may be highly poisonous to some individuals and should never be applied by an operator unprotected by a good box gas-mask.

This book however is packed tight with all kinds of useful information, and not the least valuable parts are those devoted to words of warning about undesirable methods, built up on inadequate knowledge and which may more than defeat their purpose..

There are good descriptions and illustrations of machinery. and a great number of formulae are given for insecticides and baits. A *sine qua non* for a book of this type —a good index—is included. The authors express some diffidence as to the arrangement of their material but bearing in mind the complicated way in which the subjects in reality dovetail into each other, it is a matter of surprise to see what an orderly array they have made of what must have seemed unrelieved chaos when they began their task. It is inevitable of course in a résumé of this type that the specialist should find things to criticise in the chapters dealing with his field of work: there are bound to be some small errors of detail, errors of stress and of omission. The particular value however of the book is that it will enable the specialist to link up his own branch with others and to draw upon them for new ideas. To the beginner it will not only afford an insight into the methods employed, but should offer him much inspiration in indicating how broad is the field, and how much remains still to be done, while to the general economic entomologist it will prove a very readable text-book and a handy book of reference.

F. TATTERSFIELD.

Diseases of Greenhouse Plants. By W. F. Bewley, D.Sc. Ernest Benn. Ltd., 1923, pp. 208, figs. 47. Price 12*s*. 6*d*.

A text-book on plant pathology should cover the ground embraced by the three main branches of the subject, namely diagnosis, aetiology and therapeutics. Viewing plant-pathology as an applied science the most important branch is the last named, since the very *raison d'être* of the science lies in its ability to control plant-disease. Inasmuch as any satisfactory method of control is necessarily based on a thorough understanding, not only of the host-plant and the parasitic organism, but also of the inter-relationships of the two, it is not surprising that this aspect of the science has been the latest to be developed. Treatment, it is true, was freely offered in the past, but, from the grower's standpoint it was often painfully ineffective.

Although the taxonomic side is naturally predominant in the early stages of such a science as plant-pathology, this country has been noteworthy in having had for its exponents such thorough-going systematists as Berkeley, Cooke and Massee. The explanation of this doubtless lay in the difficulty the ordinary botanist experienced in identifying the fungi concerned, and later, in the official connection of Kew with the old Board of Agriculture.

The next phase to be developed—the detailed study of the life-history of the parasite and of the susceptibility or otherwise of the host—commenced as an epoch under the inspiring genius of Marshall Ward; and its supreme importance is manifest in the fact that this line of research is still being eagerly and successfully pursued. It was reserved until a slightly later period for research on remedial measures to be taken up seriously in these islands. Without in any way slighting the experiments of previous investigators this period may be said to have commenced with the appointment of E. S. Salmon, to Wye College, in 1906, and with the establishment by the Department of Agriculture for Ireland of a field laboratory in the wilds of Connemara for G. H. Pethybridge. These two workers, set out, amongst other things, to *control* American Gooseberry Mildew and Potato Blight respectively, and it is not surprising that their problems led them into the realms of chemistry, physics and biochemistry and entailed studies of the life-history of the parasite, its biological forms and its reaction to weather-conditions, to say nothing of attention to the host-plant, its cultivated varieties, suitability to soil and climate, characters as to cropping, and varying powers of resistance to disease. With the initiation of this phase the establishment of phyto-pathology on its own footing (as a science) distinct from mycology was complete. Subsequently its importance has become fully recognised, and a many-branched state-aided, phyto-pathological service has now been organised for this country.

In the campaign against diseases under glass, no worker has specialised to the same extent as Dr W. F. Bewley, or secured such eminently successful results in all aspects of the subject. In the book now published he gives from first-hand experience the methods of combating fungus diseases existing in the sea of glass-houses lying in the Lea Valley. The book though strictly scientific is written primarily for the grower. Out of its nine chapters, fungi as such are dealt with in three only, the remaining chapters being occupied with such subjects as plant-hygiene, physiological troubles, bacterial diseases, mosaics, fungicides and steam sterilisation of soil. In addition to dealing at length with the diseases of Tomato and Cucumber it includes particulars of those of the Rose, Arum, Carnation, Chrysanthemum and other greenhouse plants. The author however is seen at his best in dealing with the major diseases of Tomatoes and Cucumbers, and no one has done more than he to elucidate their biology or to discover means of suppression.

We can confidently recommend the book to every grower as being the work of a thoroughly up-to-date scientific investigator, whose sound judgment and practical experience enable him to give the exact type of information required. It is equally essential to every pathologist and pathological library. Although not including such items as formal diagnoses and synonymy, and possessing only a short bibliography, it contains much first-hand and valuable information not found in any other book.

Should a second edition be called for a few corrections are necessary, the most important of which is the statement that *Septoria lycopersici* has not been recorded in Britain. The fungus occurred in several stations in England in 1907 (specimens being preserved at Kew) and it was for several years a scheduled disease under a D.I.P. Order of the Board of Agriculture. There is evidence also that the Index and especially the Bibliography were prepared in haste.

A. D. COTTON.

TREASURER'S STATEMENT FOR THE YEAR ENDING 31 DECEMBER, 1923

CASH ACCOUNT

Cr	£	s.	d.	*Dr*	£	s.	d.
Jan. 1: Cash at Bank . . .	89	7	0	Postage	9	5	0
Dec. 31: Subscriptions				Stationery and Minor printing	6	7	5
A. Current	242	16	0	Treasurer	192	13	4
B. Arrears	14	11	0	Secretary	2	18	9
C. Advances	21	7	3	Balance at bank	88	7	10
Entrance fees	7	17	6	On deposit at bank	150	0	0
Contributions to cost of papers in *Annals*	58	8	0				
Composition fee	14	10	0				
Bank Interest		15	7				
Total	449	12	4		449	12	4

Audited and found correct: WM. B. BRIERLEY.
R. STENTON.

21 *January* 1924.

BALANCE SHEET

LIABILITIES	£	s.	d
Subscription in advance . . .	21	7	3
Liability on *Annals*, Vol. x	170	6	2
Stock	67	15	8
Cash balance . .	101	18	5
Total	361	7	6

ASSETS	£	s.	d.	£	s.	d.
A. CASH						
Current a/c	88	7	10			
Deposit a/c	150	0	0			
Subscriptions in arrears considered good . . .	49	4	0			
Contributions owing on papers in *Annals*, Vol. x	6	0	0			
Total cash				293	11	10
B. STOCK (estimated value of stock of *Annals of Applied Biology* with publishers						
61 cases at 2*d*. each . . .		10	2			
28 bound vols. at 4*s*. each .	5	12	0			
Unbound vols. complete sets at 1s. each						
Vol. 1 156 copies	7	16	0			
2 152 ,, ...	7	12	0			
3 147 ,, ...	7	7	0			
4 135 ,, ...	6	15	0			
5 197 ,, ...	9	17	0			
6 103 ,, ...	5	3	0			
7 74 ,, ...	3	14	0			
8 70 ,, ...	3	10	0			
9 70 ,, ...	3	10	0			
10 77 ,, ...	3	17	0			
Unbound odd parts						
315 at 2*d*. each	2	12	6			
Total stock				67	15	8
				361	7	6

The sum of £400 was invested in 1922 in Savings Certificates.

Audited and found correct: WM. B. BRIERLEY.
R. STENTON.

21 *January* 1924.

VOLUME XI JULY, 1924 No. 2

OBSERVATIONS ON THE IMPROVEMENT OF POOR PASTURE IN THE WEST RIDING OF YORKSHIRE

By J. C. LYNN, M.Sc. (Agric.),

and D. ANDERSON LYNN, B.Sc. (Lond.), N.D.A. (Hons.).

(With 6 Text-figures.)

The district in which the following observations were made comprises the northern portion of the West Riding, and is a tract of country of about 1100 square miles, with Skipton-in-Craven as its approximate centre. It is a hilly area, consisting for the most part of rough pasture and moorland, intersected by the four fertile valleys of the upper reaches of the Nidd, Wharfe, Aire, and Ribble.

The two main types of geological formation are Millstone Grit and Mountain Limestone. The greater portion of the eastern part of the district lies on the former, through which, however, appear numerous outcrops of Limestone, and on which, also, are tracts of River Alluvium and Boulder Clay. Westward and to the north of Skipton the main formation is Mountain Limestone. The bulk of the Boulder Clay being detritus from Millstone Grit, Mountain Limestone and Yoredale beds, the Boulder Clay soils are not so inherently short of lime as are those on Millstone Grit.

The average annual rainfall in Skipton is 38 ins., but much of the rough pasturage on the hills around is subjected to a rainfall considerably heavier.

The principal object in the following studies was to ascertain the most efficient methods of improving the various types of pasture situated in this district (with the exclusion of moorland pasture); and the changes in the nature of the herbage induced by the various methods of treatment. It is not suggested that the conclusions arrived at can be applied indiscriminately elsewhere.

The botanical analyses were made[1] during the summer of 1922. The previous summer was exceptionally dry, and many of the pastures became parched; whereas, with the exception of May, the summer of

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[1] After the manner described by Armstrong (*Journ. Agric. Sci.* Dec. 1907).

1922 was exceptionally wet. As so often happens after a dry year, clover was unusually abundant in 1922; and this fact should be borne in mind when the analyses are being read.

The grass-land in the district can be grouped conveniently into three classes, as follows:

I. High-lying rough pasture.
II. Better-class pasture.
III. Moorland pasture.

The present studies are confined to I and II only.

I. HIGH-LYING ROUGH PASTURE.

The type of pasture referred to under this heading is, though not moorland, frequently bounded by moor-pastures growing little but heather and bilberry. Many of the fields are intakes from the moors. Such pasture may be classed under two headings as follows:

(1) White Bent land.
(2) *Agrostis* pasture.

(1) *White Bent land.* There are very large areas of upland pasturage in the West Riding of Yorkshire where the chief constituent of the herbage is White Bent (*Nardus stricta*). Generally speaking, such pastures occur on the Millstone Grit formation, but they are to be found also on gritty drifts of Boulder Clay. With White Bent is invariably associated a mat (or "fogg") which varies in thickness from 2–4 ins. On the better types of White Bent land *Festuca ovina* forms an appreciable proportion of the herbage, and as the percentage of this grass increases, so also does the value of the pasture improve. With *Nardus* is usually associated Heath Bedstraw (*Galium saxatile*), Field Wood Rush (*Luzula campestris*), and Tormentil (*Potentilla tormentilla*). In the damper types of such land various species of Carex establish themselves, and Flying Bent (*Molinia caerulea*) and Stool Bent (*Juncus squarrosus*) become prominent.

It is well known that the formation of mats is due to sour soil-conditions, which interrupt the work of those bacteria which are responsible for the decomposition of dead organic matter. When alkalinity is restored by the application of lime, and the soil once more becomes suited to the activities of such bacteria, the mat is decomposed and absorbed into the soil. Better grasses and clovers have then a chance to compete against the coarser plants, which alone can tolerate a sour and matted condition of the soil. It is interesting to note that in the majority of cases (*vide post*) lime will not only remove the mat and induce a growth of better grasses, but that it will produce, in addition, a luxuriant growth of Wild White Clover.

The removal of the mat and the better soil-conditions obtained after liming do not in themselves seem to be adequate reasons for the remarkable growth of clover that so frequently results from liming on such soils; and it has been suggested that the mat itself might contain a sensible percentage of phosphates which, on its decomposition, would be liberated in the soil, and become available as plant-food. In November 1921 three turves, each 1 foot square, and having a 3 in. mat, were cut from a rough White Bent pasture, overlying Millstone Grit. These were analysed by the Department of Agricultural Chemistry, University of Leeds, with the following results:

	% N_2	% P_2O_5	% moisture	Loss on ignition	Weight in grams per sq. ft.
Sod A	1·98	·220	8·8	68·46	1050
„ B	1·75	·220	7·53	60·02	1030
„ C	2·09	·216	10·79	68·64	613

It will be seen that such a mat contains an appreciable amount of nitrogen and phosphoric acid. The latter, in fact, on the average analysis of the above three turves, is equivalent to a dressing of 12 cwts. of 30 per cent. basic slag per acre, assuming the phosphates present to be of value equal to those present in slag; and it is reasonable to suppose that, although the available phosphates may form only a small percentage of the total phosphates liberated, they nevertheless have a considerable influence on the growth of the clover which usually follows the decomposition of such mats.

It is to be observed that mats on White Bent land are particularly tough and fibrous, and that there is no admixture of soil with them. The soil under such mats will be found, even after continuous heavy rains, to be quite dry.

A number of cases of the improvement of White Bent land have been studied, three typical examples of which are here cited.

Case A. A rough pasture, 25 acres in area, sloping to the south, and dry. Soil, a sandy loam, overlying Millstone Grit. Altitude, 600 ft. The untreated portion of the field is covered by a mat, 2–3 ins. in thickness, of undecomposed vegetable matter; and there is a lime-requirement (as determined by the Hutchinson-Maclennan method) of 41 cwts. per acre. This area is valued by the tenant at 5*s*. per acre rent.

A portion of the field, 6 acres in area, was limed in 1874. at the rate of about 5 tons per acre of cob lime. This plot stands out conspicuously as a bright green patch in the distance, and on close inspection it is found that the mat or skin has disappeared and the ground is firm to the tread. It has a lime-requirement of 10 cwts., and is valued by the tenant at 15*s.* per acre rent.

To a strip down the middle of the limed area high-grade slag was applied ten years ago at the rate of 10 cwts. per acre. The effect of the slag on this strip is very striking, the herbage showing up as distinctly greener than the "limed only" area, and the clover being more conspicuous and more vigorous. Stock are continually working on this slagged portion, which is consequently always bare. At the same time a portion of the unlimed area received a similar dressing of slag; but, on examination and analysis in 1922, the herbage on this portion showed no differentiation from that on the untreated part of the field.

In February 1922, 30 per cent. slag was applied at the rate of 10 cwts. per acre to a plot one-tenth of an acre in area on the limed portion, and extending also over the unlimed portion for another one-tenth of an acre. When the plot was visited six months later, in August 1922, the effect of the slag on the limed area was already very marked; and the clover in it even more vigorous than in that slagged ten years ago; whereas on the unlimed area no result could be observed. The tenant values the limed-and-slagged area at 20*s.* per acre rent.

The differences in herbage induced by these various methods of treatment is evident from the accompanying table. For comparative purposes it was decided to group the constituents of the herbage into four classes, as follows:

(1) Leguminosae.
(2) Comparatively useful grasses.
(3) Comparatively useless grasses.
(4) Weeds (and bare space).

It is realised that any division of grasses into classes such as the above can at best be but arbitrary, and a great deal of latitude must be allowed to the terms "Comparatively Useful" and "Comparatively Useless." Although, for instance, *Molinia* is grouped in Class 3, it is none the less one of the most valuable grasses in the summer months on certain types of unimproved hill-land. Compared with *Nardus*, it is a useful grass; compared, however, with the better-class grasses such as the Fescues, it is an inferior grass, and is hence classed as such. After a lengthy consideration and two years' observation of the grazing of the plots, the grasses were grouped as below, and this classification has been adhered to throughout the series of analyses, so that the diagrams constructed therefrom might have some comparative value.

Botanical Analysis of Case A, 2nd August 1922.

	Untreated	Limed 1874	Limed 1874 Slagged 1922
Species	%	%	%
Leguminosae:			
Trifolium repens	—	13·5	24·8
Lotus corniculatus	—	4·0	—
Comparatively useful grasses:			
Festuca ovina	16·3	25·1	29·4
Phleum pratense	—	·8	·4
Cynosurus cristatus	—	2·8	3·2
*Poterium sanguisorba	—	·8	·2
*Plantago lanceolata	—	·5	—
Comparatively useless grasses:			
Nardus stricta	45·4	11·7	5·9
Agrostis sp.	10·3	17·9	13·7
Holcus lanatus	·4	7·6	6·9
Anthoxanthum odoratum	·3	·2	·3
Molinia caerulea	3·5	—	—
Triodia decumbens	·9	·3	1·4
Poa compressa	·2	—	·3
*Juncus squarrosus	2·4	—	—
Weeds:			
Galium saxatile	9·4	1·7	—
Potentilla tormentilla	5·2	1·4	1·0
Viola sp.	—	·7	·7
Ranunculus sp.	—	Trace	·7
Prunella vulgaris	—	1·4	1·8
Euphrasia officinalis	—	·9	1·0
Veronica sp.	—	Trace	—
Bellis perennis	—	·2	·2
Polygala vulgaris	·3	Trace	·1
Aira caespitosa	Trace	Trace	—
Luzula campestris	3·3	3·5	2·3
Carex sp.	—	1·0	2·6
Moss	·5	1·2	1·4
Leguminosae	Nil	17·5	24·8
Comparatively useful grasses	16·3	30·0	33·2
Comparatively useless grasses	63·4	37·7	28·5
Weeds	18·7	12·0	11·8
Totals	98·4	97·2	98·3

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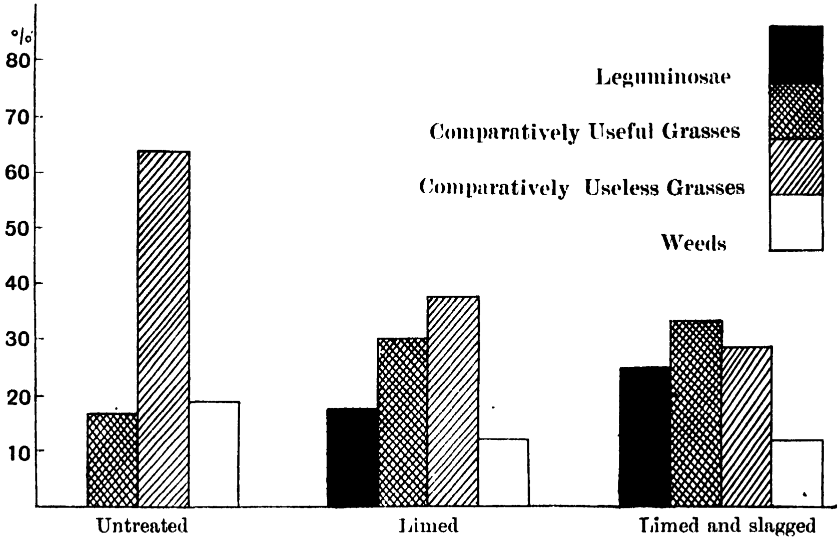
* For purposes of convenience, throughout the tables, *Poterium*, *Plantago* and *Achillea* have been included in the "Comparatively useful grasses," and *Juncus squarrosus* in the "Comparatively useless grasses."

The results of the analysis may be illustrated diagrammatically as follows:

Diagram to illustrate analysis of Case A.



While the botanical analysis was being made, it was observed that, although liming had not entirely eradicated it, the *Nardus* on the limed and limed-and-slagged areas was interspersed with other plants, its tufts being quite small, much finer, and in many cases grazed almost as closely as the surrounding herbage. Much of the *Nardus* had been pulled out by the roots by stock, and evidently discarded, for it was lying about on the surface in small, dead tufts. *Agrostis* was closely eaten-down on the treated portions of the field, where it was growing in association with *Cynosurus*, *Festuca*, and *Trifolium repens*. On the untreated area it was growing in coarse tufts, and was ungrazed and altogether different in appearance from that on the improved land. On the latter, *Agrostis* was undoubtedly preferred by stock to *Holcus* and *Anthoxanthum*—in fact it was eaten as readily as any other grass growing on the treated area. *Triodia* was very little touched by stock.

Case B. A rough pasture, an "in-take" from the neighbouring heathered moor, 80 acres in extent. Altitude, about 600 ft. rising to 800 ft. Soil, variable, but on the lower half, including the improved portion, a rather light loam formed from a gritty drift of Boulder Clay overlying Milltone Grit. Southern aspect. The lower half is fairly dry, the upper is more level, and wet, and covered with several inches of black peat. Since the upper portion differs markedly in both physical character and botanical composition from typical White Bent land, the following observations are confined to the lower half only. The whole field was valued in 1902 at 5*s*. per acre rent.

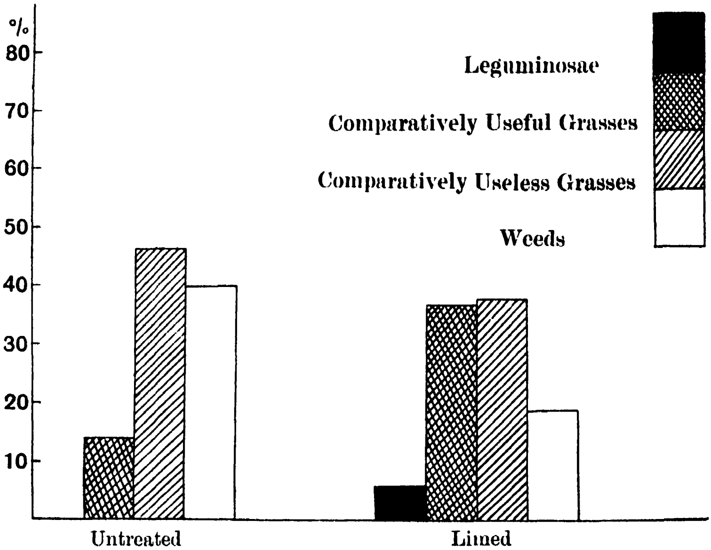
Although on the untreated dry area there are a few damp spots formed by springs emerging from the hillside, where *Juncus communis* appears, the greater part of the area is naturally dry. There is a mat of from 1½–2 ins. in thickness. To a rectangular plot of 6 acres, cob-lime was applied at the rate of 6 tons per acre about the year 1862. This area standing out clearly from hilltops eight miles away as a bright green patch, and also being closely grazed, forms a marked contrast with the rest of the field. The herbage is, however, not so good as it was ten years ago. A mat is now beginning to form again and some of the coarser grasses and weeds to reappear; but the following analysis will indicate the improvement maintained after 60 years:

Botanical Analysis of Case B, 18th July 1922.

Species	Untreated %	Limed 1862 %
Leguminosae:		
Trifolium repens	—	5·7
Comparatively useful grasses:		
Festuca ovina	14·0	36·1
Dactylis glomerata	—	·4
*Achillea millefolium	—	·3
Comparatively useless grasses:		
Nardus stricta	32·0	—
Agrostis sp.	9·5	26·8
Holcus lanatus	1·0	5·7
Anthoxanthum odoratum	·4	1·2
Molinia caerulea	1·0	—
Triodia decumbens	·3	—
Poa compressa	2·0	4·2
Weeds and bare space:		
Galium saxatile	13·0	9·1
Potentilla tormentilla	3·3	—
Euphrasia officinalis	Trace	Trace
Rumex acetosa	·2	·7
Cnicus arvensis	—	·5
Polygala vulgaris	Trace	—
Aira caespitosa	9·0	·2
Juncus communis	7·0	—
Luzula campestris	3·9	6·8
Carex sp.	·1	Trace
Bare space	3·3	1·4
Leguminosae	Nil	5·7
Comparatively useful grasses	14·0	36·8
Comparatively useless grasses	46·2	37·9
Weeds and bare space	39·8	18·7
Totals	100·0	99·1

* See footnote to first table.

Diagram to illustrate analysis of Case B.



The difference in the quality of the *Agrostis* growing on the untreated and on the limed areas was very noticeable; that on the former being ungrazed, brown and tough; that on the latter being closely eaten down, green, sweet smelling, and evidently much favoured by stock. The clover appeared to be losing vigour, and was undoubtedly present in greater quantity 10 or 15 years ago.

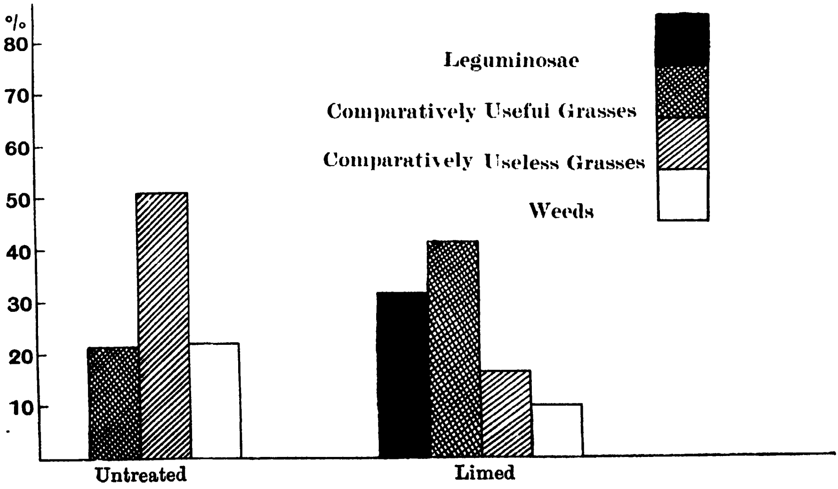
Case C. A rough pasture 24 acres in area. Altitude, 600 ft. Situated on a southern slope. Soil, a gritty loam, overlying Millstone Grit, and dry. A portion of the field, 5 acres in area, was limed in 1911, at the rate of 5 tons per acre small lime. At a distance this plot could be clearly distinguished from the untreated part of the field by its fresh green colour. On the untreated area the grass was 8–10 ins. long, brown and stemmy, interspersed with patches of rushes; and there was a mat 2–3 ins. in thickness. The limed plot on the other hand was, as usual, closely grazed, the rushes had disappeared, as also had the mat. The rental value of the unimproved land was estimated by the tenant at 5*s.* per acre, that of the improved at 25*s.* Another part of the field was limed in March 1921, and in August 1922 a difference was already apparent in the herbage, and clover was appearing. It was noticeable that stock already preferred this newly-limed area to the untreated.

Botanical Analysis of Case C, 14th August 1922.

Species	Untreated %	Limed 1913 %
Leguminosae:		
Trifolium repens	—	23·0
Lotus corniculatus	—	8·3
Comparatively useful grasses:		
Festuca ovina et duriuscula	20·8	32·0
Dactylis glomerata	—	1·1
Phleum pratense	—	·9
Poa pratensis	—	1·3
Cynosurus cristatus	·5	4·1
Trisetum flavescens	—	1·1
*Plantago lanceolata	—	1·0
Comparatively useless grasses:		
Nardus stricta	37·2	·8
Agrostis spp.	7·1	11·0
Holcus lanatus	4·3	2·6
Anthoxanthum odoratum	·6	·6
Triodia decumbens	1·8	1·2
Weeds:		
Galium saxatile	·7	—
Potentilla tormentilla	12·8	·5
Euphrasis officinalis	—	·5
Rumex aceotsa	·9	—
Ranunculus sp.	—	·4
Hieracium pilosella	—	·7
Prunella vulgaris	Trace	·2
Viola sp.	—	1·2
Cerastium vulgatum	—	·4
Aira caespitosa	1·9	·8
Juncus communis	2·5	—
Luzula campestris	6·0	2·4
Carex sp.	2·1	2·2
Moss	·1	·5
Leguminosae	Nil	31·3
Comparatively useful grasses	21·3	41·5
Comparatively useless grasses	51·0	16·2
Weeds	27·0	9·8
Totals	99·3	98·8

* See footnote to first table.

Diagram to illustrate analysis of Case C.



Throughout the district a number of other cases of the treatment of White Bent land have been observed, and the results recorded are in entire agreement with those cited in the foregoing examples. Owing to lack of space, the briefest details only can be furnished of three further typical instances, as under:

Case D. A rough pasture covered with a 1½–2 in. mat, growing mainly *Nardus*, *Galium* or *Potentilla*, with an admixture of *Festuca ovina et duriuscula* but no *Trifolium repens*. Soil, medium loam, overlying Mountain Limestone; fairly dry. Six tons per acre of cob-lime applied in 1912 to a portion of the field has removed the mat, eradicated the *Nardus*, induced the growth of clover, and increased the rental value from 5*s*. to 25*s*. per acre.

Case E. A high-lying, exceedingly rough pasture. Soil, heavy loam overlying Millstone Grit. Three and a half tons of small lime per acre applied to part of the field in 1912 has converted a pasture worth about 1*s*. per acre into one worth 20*s*. It is interesting to note that one of the chief constituents of the improved herbage is *Agrostis*.

Case F. A rough pasture. Soil, medium loam, overlying Millstone Grit, covered with a 1½–2 in. mat, and growing mainly *Nardus*, *Festuca ovina*, *Galium* and *Luzula*. High-grade slag applied at the rate of 10 cwts. per acre to part of the field in 1915 has produced no evident result. Gas-lime applied to another portion of the field, has largely eradicated the *Nardus* and *Galium* (which have been replaced mainly by *Festuca ovina*), and has produced a sweet, green pasture.

It will be noticed that, with the exception of Case E, the dressing of lime in the above examples was 5 tons per acre or more. In such cases a layer of lime is found from 3–6 ins. below the surface of the soil. From this fact and the study of Case E, together with general observations, there appears to be good reason to believe that, even on the roughest pastures, so moderate a dressing as 3 tons per acre of good quality cob-lime is sufficient to break up the mat, to effect a valuable improvement in the herbage, and to prepare the way for an excellent result from slag. It is possible that this point will be demonstrated by experiments which were laid down by one of the authors in the Skipton district in 1923, under the auspices of Leeds University.

The foregoing studies appear to justify the following conclusions in regard to the treatment of White Bent land:

(1) A big and lasting improvement can be effected on such land, if reasonably dry, by suitable applications of lime.

(2) Slag applied after lime will still further improve such land.

(3) Slag without lime will effect no improvement.

(2) *Agrostis pasture.* Pastures in which the chief constituent of the herbage is *Agrostis* occupy considerable areas in the West Riding. They are found overlying Mountain Limestone, Millstone Grit, River Alluvium and Boulder Clay formations. With *Agrostis* are usually associated *Festuca ovina*, *Galium*, *Potentilla* and *Luzula*. Such pastures are usually matted, but, in the districts under consideration, *Agrostis* mats are not generally so thick as those associated with *Nardus*. It is to be noted also that *Agrostis* mats are as a rule more open in texture, and often have a considerable amount of soil admixed with them; they are consequently not so impenetrable to rain and to the roots of plants as are mats of White Bent.

The kind of treatment to which *Agrostis* pasture responds depends largely on the thickness and texture of the mat, together with the lime requirement. In some cases basic slag alone will effect a satisfactory improvement, whereas in others a previous dressing of lime is essential. Dr Hanley (Leeds University) informs us that, in his experience, basic slag is generally effective on such pastures where the lime-requirement is less than 30 cwts. per acre; that its action is doubtful where the lime-requirement lies between 30 and 40 cwts.; that it is not effective when the lime-requirement exceeds 40 cwts.; and that from an inspection of the mat and a knowledge of the lime-requirement, it is often possible to determine whether slag will be effective or not. The following two cases are illustrative of these remarks:

Case G. An old pasture covered with a 1½ in. mat, and with a lime-requirement of 30 cwts. per acre. Soil, a heavy loam inclined to be damp, derived from Millstone Grit Shale. Altitude, 850 ft.

In February 1920 three plots, each half an acre in area, were laid down by the University of Leeds, as follows:

Plot 1. Lime 2 tons per acre.

Plot 2. Basic slag, 38 per cent., 10 cwts. per acre.

Plot 3. Untreated.

In April 1920 a cross dressing of 4 cwts. per acre of Kainit was applied over one-third of the plots; and in November 1921 a cross dressing of basic slag, at the previous rate, was applied over another one-third[1].

Frequent visits have been made to these plots since January 1921. It was noticed that the basic slag plot was the first to show differentiation, the percentage of clover in it visibly increasing during the summer of 1921, and still more so in 1922; and it was more closely grazed than the other plots. In August 1922, when the analysis was being made, the contrast between this plot and the others was very marked; it was eaten bare by stock, the greater part of the mat had disappeared, and the clover looked particularly healthy and vigorous and was spreading rapidly. The limed plot, on the other hand, had not been so well grazed, was still considerably matted, and contained but little clover. It is probable that the lime had not yet had sufficient time to produce anything like its maximum effect. *Agrostis* on the limed and untreated areas was not much grazed by stock, although on the slagged plot it had been readily eaten down.

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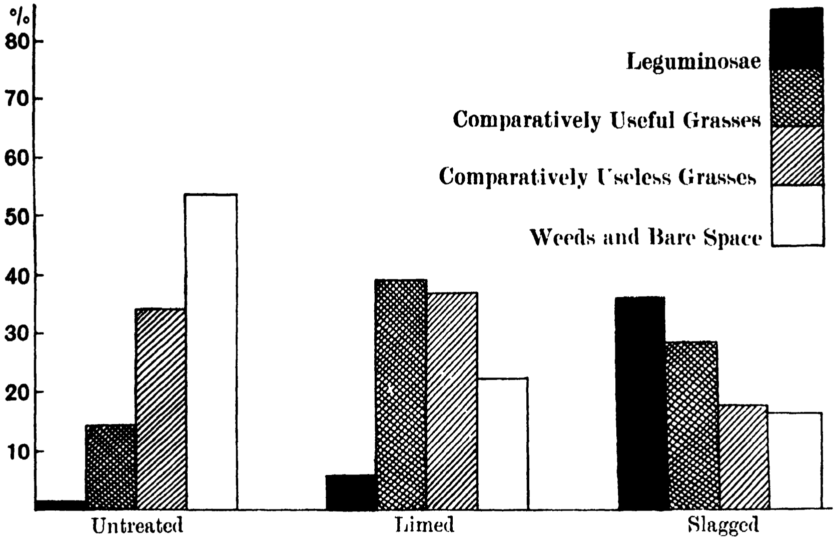
[1] See *Guide to Grassland Demonstrations in Yorkshire.*

Botanical Analysis of Case G, 19*th August* 1922.

Species	Untreated %	Limed 1920 %	Slagged 1920 %
Leguminosae:			
Trifolium repens ...	·9	4·8	33·5
Trifolium pratense ...	—	Trace	—
Lotus corniculatus ...	·5	·8	2·5
Comparatively useful grasses:			
Lolium perenne ...	1·1	—	·6
Festuca ovina ...	9·0	28·6	15·2
Dactylis glomerata ...	—	5·0	—
Phleum pratense ...	—	2·0	2·0
Cynosurus cristatus ...	2·2	3·1	7·2
*Plantago lanceolata ...	2·0	·5	3·6
Comparatively useless grasses:			
Nardus stricta ...	—	1·1	·5
Agrostis spp.	26·1	27·3	16·0
Holcus lanatus ...	2·5	1·4	—
Anthoxanthum odoratum	·7	·9	·1
Triodia decumbens ...	·9	—	1·1
Briza media	·2	—	—
Weeds and bare space:			
Galium saxatile ...	—	·4	—
Potentilla tormentilla ...	2·7	—	—
Rumex acetosa ...	·7	1·0	—
Ranunculus acris ...	1·8	1·2	·3
Hieracium pilosella ...	—	·9	·9
Leontodon sp. ...	5·6	3·0	2·0
Bellis perennis ...	1·1	—	1·0
Centaurea nigra ...	·7	—	—
Prunella vulgaris ...	—	—	·5
Campanula rotundifolia	2·7	—	—
Aira caespitosa ...	4·7	4·0	·7
Luzula campestris ...	32·2	3·3	3·5
Carex sp.	1·3	·8	—
Moss	—	3·0	2·2
Bare space	—	4·9	5·0
Leguminosae	1·4	5·6	36·0
Comparatively useful grasses	14·3	39·2	28·6
Comparatively useless grasses	30·4	30·7	17·7
Weeds and bare space ...	53·5	22·5	16·1
Totals ...	99·6	98·0	98·4

* See footnote to first table.

Diagram to illustrate analysis of Case G.



Analyses were made of the plot which received only the initial dressing of slag in 1920, and of the lime-alone plot; and the preceding table indicates the differences in herbage induced by these dressings. An analysis of the untreated area made by Miss L. I. Scott, B.Sc., Leeds University, in 1921, is given above; and, although it is realised that analyses made in different seasons are not strictly comparable, it is felt that these figures have some value as indicating the original nature of the herbage.

Case H. A rough hill pasture 21 acres in area, covered by a 2 in. mat and sloping steeply to the south. Mean altitude, 850 ft. Soil a good loam, varying in depth from 1–2 ft., immediately overlying limestone, and dry. In 1908 small lime was applied to 7 acres of this field, in parallel strips running across the slope, at the rate of 6¾ tons per acre. The cost of the lime was then 3*s.* 9*d.* per ton.

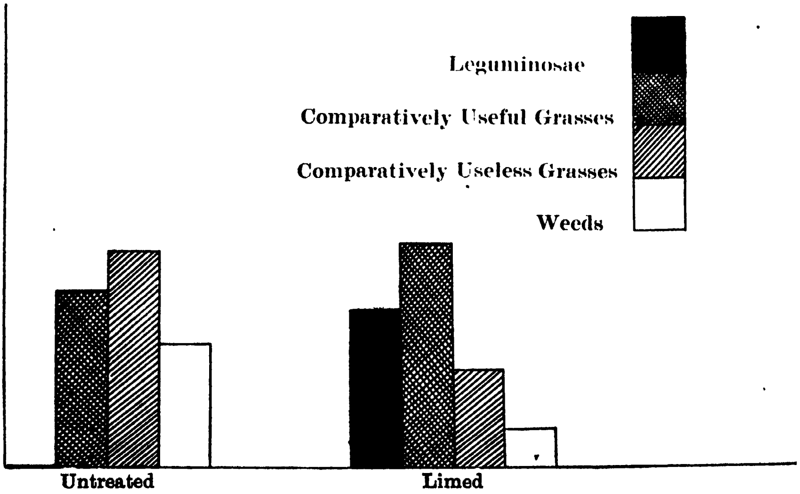
In August 1922 the untreated area was red with the flowering heads of *Agrostis*, whereas on the limed strips few flower stalks could be found, and the herbage was sweet and closely grazed. As in other cases, the *Agrostis* persisting on the limed portions seemed to possess an altogether superior economic value to that on the untreated land. The tenant values the limed area at 22*s.* 6*d.* per acre rent and the unlimed at 7*s.* 6*d.*

Botanical Analysis of Case H.

Species	Untreated %	Limed 1908 %
Leguminosae:		
Trifolium repens ...	—	16·8
Lotus corniculatus ...	·1	12·9
Comparatively useful grasses:		
Lolium perenne ...	—	1·8
Festuca ovina et duriuscula	32·0	35·9
Cynosurus cristatus ...	-	1·0
*Achillea millefolium ...	1·4	3·8
Comparatively useless grasses:		
Agrostis spp.	34·8	10·7
Holcus lanatus... ...	·4	4·3
Anthoxanthum odoratum	2·8	3·0
Triodia decumbens ...	3·1	·8
Weeds:		
Galium saxatile ...	12·9	—
Galium pusillum ...	—	1·8
Potentilla tormentilla ...	5·5	·1
Rumex acetosa ...	·4	—
Ranunculus sp. ...	—	·4
Cnicus arvensis ...	—	Trace
Prunella vulgaris ...	—	·1
Campanula rotundifolia ...	-	·2
Euphrasia officinalis ...	—	Trace
Viola sp.	·2	1·4
Thymus serpyllum ...	—	·1
Aira caespitosa ...	-	·3
Luzula campestris ...	4·1	1·7
Carex sp.	·3	·8
Moss	—	·2
Leguminosae	·1	29·7
Comparatively useful grasses	33·4	42·5
Comparatively useless grasses	41·1	18·8
Weeds	23·4	7·1
Totals ...	98·0	98·1

* See footnote to first table.

Diagram to illustrate analysis of Case H.



Brief notes of other examples of the treatment of *Agrostis* pasture are herewith presented:

Case J. A field with a 1–1½ in. mat, on medium grit, growing mainly *Agrostis* with *Galium*, *Potentilla*, *Festuca ovina* and a little *Cynosurus*. Cob-lime at the rate of 5 tons per acre applied to a part of the field in 1919 has eradicated the mat, consideraby decreased the *Agrostis*, and induced a healthy growth of clover. Another area received 12 cwts. per acre of 35 per cent. basic slag, which has produced a similar but less marked improvement.

Case K. A poor pasture with a 1½ in. mat. Soil, a good loam, 12–18 ins deep, overlying Mountain Limestone. Herbage mainly *Agrostis* intermixed with *Festuca ovina* and *Galium*. High-grade slag applied at the rate of 10 cwts. per acre in 1913 has almost eradicated the mat, induced the growth of *Trifolium repens*, *Cynosurus*, and a small quantity of *Lolium perenne*. A large quantity of *Agrostis* still remains, but the grass now most in evidence is *Festuca ovina*. This field gives one the impression that, although slag has effected a big improvement, it has been hindered in its action by the deficiency in lime and the presence of the mat; and that a better result would have been obtained had there been a previous light dressing of lime.

Case L. A poor pasture with a 1–1½ in. mat, growing chiefly *Agrostis*, *Festuca ovina*, *Galium*, *Potentilla* and *Luzula*. Soil derived from Millstone Grit. Applied to certain areas in 1917 and to others in 1919, small lime at the rate of 25 cwts. per acre has produced a marked improvement. Even this light dressing has eradicated the mat, and although clover is not prominent, small starved plants are present everywhere. In December 1922 there was every indication that a dressing of slag would produce excellent results.

The evidence presented by the foregoing studies is somewhat conflicting, and shows that the treatment of *Agrostis* pasture is not so straightforward as that of White Bent land. While it is realised that further work is required before any definite conclusions can be drawn, our observations tend to confirm Dr Hanley's opinions as quoted on p. 145. Where the mat does not exceed 1½ ins. in thickness basic slag will effect an improvement, which may be either inferior (see Case J) or superior (see Case G) to that produced by lime alone. Where the mat is as thick as 2 ins., even on soil overlying limestone, lime will produce a satisfactory result.

II. BETTER-CLASS PASTURE.

Under this heading may be included those lower-lying and productive tracts of land which are found in the valleys and on the lower slopes of the hills of the West Riding. Such fields occur commonly on River Alluvium, on the Mountain Limestone formation where there is a sufficient depth of good soil, on Boulder Clay, and, less frequently, on Millstone Grit. Briefly speaking, applications of basic slag seldom fail to effect improvement on the heavier and damper soils; whereas on drier soils, inclined to be sandy, steamed bone flour is usually more successful. The fact that on medium and lighter land basic slag is not always efficacious may, in many cases, be attributed to the deficiency in lime occasioned by the heavy rainfall of the district. Lack of lime in such fields is indicated by the presence of a mat, which in good pastures may vary from a trace only observable here and there on the rougher portions of the ground, to 2 ins. in thickness on poorer fields. As a rule, where the mat exceeds 1½ ins. in depth, an initial light dressing of lime is required, which may be followed by the usual application of slag. Slag alone, without lime, sometimes fails to evoke any response whatever. A striking example of the efficacy of lime in improving a pasture which, although not of the best, was still far from being poor, is herewith described.

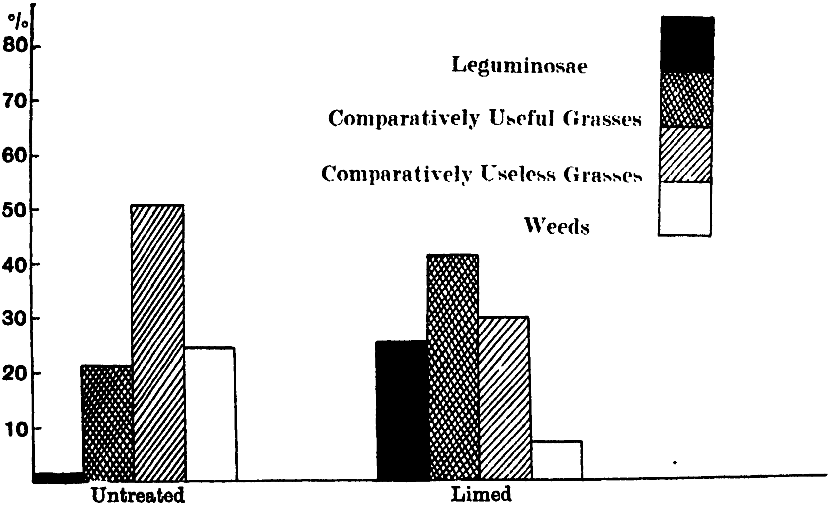
Case M. A field of 70 acres, on a south-eastern slope. Altitude, 400 ft. Soil, a medium loam, derived from a gritty drift of Boulder Clay. Considerable variations occur in the field, the eastern area having poorer herbage and a 1–1½ in. mat. The botanical analysis which follows applies to this portion only, two acres of which was limed in 1914, at the rate of 5 tons per acre cob-lime. This application has effected a most conspicuous improvement, the herbage on the limed area starting into growth much earlier and being green and closely-grazed throughout the season. The lime-requirement of the improved area is 20 cwts. and that of the untreated 42 cwts. Other strips of this poorer part of the field have been treated with basic slag with no result. When the field was analysed it was noticed that, as in other cases, the *Agrostis* on the limed area was as closely grazed as the better grasses, green and sweet-smelling; whereas that on the untreated area was ungrazed, reddish-brown, tough and wiry.

Botanical Analysis of Case M, 15th July 1922.

Species	Untreated %	Limed 1914 %
Leguminosae:		
Trifolium repens ...	·9	25·2
Lotus corniculatus ...	·6	—
Comparatively useful grasses:		
Lolium perenne ...	·8	2·7
Festuca ovina ...	14·1	19·3
Dactylis glomerata ...	5·3	3·3
Poa trivialis	·5	9·9
Trisetum flavescens ...	·2	3·0
Cynosurus cristatus ...	—	3·0
*Achillea millefolium ...	·3	—
Comparatively useless grasses:		
Agrostis spp. ...	41·9	17·5
Holcus lanatus ...	5·5	7·0
Anthoxanthum odoratum	3·1	1·4
Weeds and bare space:		
Potentilla tormentilla ...	1·5	—
Rumex acetosa ...	6·0	1·2
Ranunculus sp. ...	·6	1·0
Cnicus arvensis ...	1·1	3·4
Aira caespitosa ...	·2	—
Luzula campestris ...	7·6	1·3
Bare space	7·4	—
Leguminosae	1·5	25·2
Comparatively useful grasses	21·2	41·2
Comparatively useless grasses	50·5	25·9
Weeds and bare space ...	24·4	6·9
Totals ...	97·6	99·2

* See footnote to first table.

Diagram to illustrate analysis of Case M.



The above case illustrates the fact that even better-class pastures in the district under consideration respond in a remarkable way to applications of lime, the increase in the growth of clover being particularly striking. It should be remarked that Case M is by no means an isolated instance of such improvement.

Our thanks are due to Dr J. A. Hanley for his kindness in taking soil-samples and furnishing lime-requirements, and for other valuable assistance; and to L. A. Thompson, M.Sc., for giving us details of his own researches into pasture-improvement in Northumberland.

SUMMARY.

There are large areas of rough hill pasture in the West Riding of Yorkshire which are covered with a fibrous mat of undecomposed vegetable matter 1–3 ins. thick, and whose herbage falls naturally into two types, according to whether *Nardus* or *Agrostis* predominates. On such land lime forms an important ameliorative agent, breaking up the mat, decreasing the *Nardus*, encouraging the finer grasses, and inducing a vigorous growth of clover.

It is shown that on reasonably dry land growing from 30–40 per cent. of *Nardus stricta*, 15 20 per cent. of *Festuca ovina*, and a conspicuous amount of *Galium saxatile*, *Luzula campestris* and *Potentilla tormentilla*, suitable applications of lime effect a striking improvement, which may be maintained for as long as 60 years; that lime followed by basic slag produces still better results; that basic slag alone has no effect.

Where *Agrostis* predominates the mat is more open in texture and usually thinner than that associated with *Nardus*. On such land a satisfactory improvement is effected in certain cases by lime; and in others, where the mat does not exceed 1½ ins. in thickness, by basic slag alone. A great change is observed in the quality and palatability of the *Agrostis* as a result of such treatment.

Even on lower-lying and better-class pastures in this district most beneficial results may be obtained from dressings of lime.

(*Received March 7th*, 1924.)

THE DENSITY OF UNICELLULAR ORGANISMS

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(With 6 Text-figures.)

In biological studies of the soil or other materials, where it is desirable to calculate the total mass of a population from quantitative data representing numbers and dimensions, an accurate knowledge of the density of the particular cell or cells concerned is of considerable importance. The literature involving studies of this type seems to indicate that the values that have been applied to this purpose are largely speculative since, in no instance noted, have they been supported by definite experimental data.

The present investigation has grown out of studies upon the capacity of the soil for the adsorption of certain unicellular organisms. In this work some of the cells mentioned in the present paper were used. The relation of the two developed as a result of the technique applied in the adsorption studies. This has been essentially as suggested by Cutler (2) earlier, and consists in shaking a definite quantity of soil with varying numbers of the cells under study and, after the suspension has settled for 3-4 minutes, in order to permit the coarser particles and floccules to fall out, counting directly the number of cells remaining in free suspension with the aid of an haemocytometer. Since in these studies only a gram or less of soil is used with but 1-2 c.c. of the suspended organisms, a knowledge of the rate of fall of the individual cells would be very useful in determining just how the sample of the supernatant liquid should be withdrawn to represent the true concentration of the cells remaining in suspension. This part of the procedure will be treated in greater detail at a later time.

In the present work, both plant and animal cells have been studied. For the former, a pure culture of unicellular alga was kindly supplied by Dr Bristol of the Mycology Department. This particular species (*Scenedesmus*, number D) was used by Bristol and Page (1) in their recent studies upon nitrogen fixation. Yeast (*S. cerevesiae* and *glutinus*) and spores of *B. mycoides* were also studied in this way. Since the latter studies are still incomplete, only the results with the algal cells will be considered in any detail at the present time. For the animal cells, the cysts of protozoa alone were studied. The cysts of *Hartmanella hyalina*, *Colpoda* sp. and *Gonostomum* sp., were used for this purpose. The first was supplied in pure culture by Mr Cutler, and the second by Mr Sandon, while the third was obtained by the writer from a sample of Utah soil supplied through the kindness of Prof. C. T. Hirst of the Utah Agricultural Experimental Station. The stock culture of alga was grown in the standard solution suggested by Bristol and Page(1) which included together with small quantities of other salts, calcium nitrate as a source of nitrogen and glucose as a source of energy. When grown upon a solid substratum, the same basic medium was used with the addition of 1·5 per cent. agar-agar. The protozoa were grown in petri dishes upon a nutrient agar substratum upon the surface of which 1–3 c.c. of water was maintained.

PROCEDURE.

It was found that the first necessity for a study of this nature was an observation cell in which, after filling and mounting upon the microscope, the suspending medium should be, as nearly as possible, devoid of all superficial movements or currents. This condition was finally obtained by the use of two heavy glass slides 4 mm. in thickness between which, on either side, two strips of glass 7·5 mm. broad and 1·2 mm. thick were sealed. This then gave a cell 9 cm. long, 1 cm. broad and 1·2 mm. in depth. To the open ends of the cell so formed, an outlet and inlet constructed of heavy capillary tubing, were sealed. Both of these parts were made as short as possible and the former drawn out to a small opening in order to reduce the exposure of the liquid to a minimum. It was found best to keep both the inlet and outlet tubes horizontal with the top of the cell. After filling, care being taken to remove all air bubbles, a solid glass tube was inserted into the opening of the inlet—a short piece of rubber tubing being previously mounted upon this part. Thus there was left exposed but the small opening at the outlet which could, when desirable, be easily protected by placing a cap over it.

After insulating the observation chamber against direct contact with metal surfaces, it was mounted upon the movable stage of a microscope which, for this purpose, was always placed in a horizontal position. Thus, there was available for observation and measurement, a distance of 1 cm. fall, with a focal depth of 1·2 mm.

In order to avoid the effect of heating from the light used, illumination was effected by the use of a 40-watt bulb suspended in a sealed, cylindrical glass jar which in turn was placed in a large square battery jar and water from the tap circulated about the whole inner compartment thus constituted. The outer jar was covered with heavy paper with the exception of a round, ground glass window immediately before the microscope. This latter permitted focusing through the cell directly into the light without the use of a condenser. With walls of such thickness, it was not possible to use a high power objective, but by using a 16 mm. Spencer lens objective and drawing the tube of the microscope to its maximum length, a magnification of about 400 diameters was obtained and proved entirely satisfactory for the measurement both of the velocity and dimensions of all cells studied.

In random measurements of individual cells for both velocity and dimensions, it was found necessary, particularly in the case of the smaller ones, to devise a method of measuring their diameter as they fell through the suspending medium towards the bottom of the observation chamber. The larger protozoan cysts, which fell rather too rapidly for accurate measurement in the manner to be described, were measured as they rested upon the bottom.

For the purpose of measuring the diameter of the smaller cells under the conditions indicated, a Spencer micrometer eye-piece (number 425), with vernier and movable scale was used. It was found possible to mount immediately about this movable scale, a simple micrometer eye-piece scale which, by virtue of its position would be stationary in relation to the movable scale. Due to its proximity to the latter, both scales were in clear focus at all times. Both the movement of the vernier and the fixed scale were calibrated against a standard stage micrometer. Thus by the use of the movable stage of the microscope, one side of the falling cell could be held to a certain position against the fixed scale in the eye-piece while the movable scale was adjusted to the other side. With the diameter registered upon the two scales as the distance between a point upon each respectively, by the manipulation of the vernier, the actual diameter in mm./1000 could be subsequently read off at leisure.

The velocity of the particle was usually ascertained prior to the measurement of its diameter. This was accomplished, with the aid of a stop-watch, as the cell fell vertically past the fixed scale. It was readily possible, however, by placing the two scales at right angles to each other to take both measurements simultaneously. Since in these measurements the tube of the microscope was fully extended and could not easily be held entirely rigid, the very slight movement which almost unavoidably accompanied the manipulation of the vernier, made the former procedure of making the measurements independently the more desirable. It might be noted that if the measurements were made simultaneously, since the whole micrometer with both scales could be rotated in the barrel of the microscope, it was possible to measure either of the apparent dimensions of an object, moving under these conditions, regardless of the direction, or within certain limits, the rate of its movement.

In the actual determination of the velocity of fall, it was found that great care was required in ascertaining the equilibrium, both in the suspending medium and in the rate of fall itself. For while two consecutive measurements of the rate for the same cell through 60 or 80 seconds may give results sufficiently close in their agreement to be accepted as reliable duplicates, not infrequently, in a longer series of successive measurements it has been found that such a disagreement, though slight, continued in a definite direction. The nature of this tendency will be made clear by reference to Fig. 1. Thus, if a series of successive measurements be plotted as time against the rates observed at each interval, it may require a considerable period before the rate of fall actually approaches a constant value and the fluctuations become truly random in nature rather than unidirectional. The direction of the change in the rate of fall is by no means always the same. Sometimes it increases to a constant rate as shown at *A* and at other times it diminishes as indicated at *B* (Fig. 1). The nature of these effects depends, seemingly, upon the extent and direction of the currents naturally imparted to the fluid mass at the time of filling the cell.

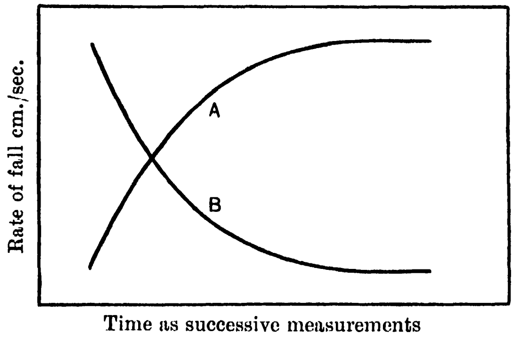

Fig. 1. Curves showing general tendency of velocity changes previous to the appearance of fluid equilibrium.

Since a rather marked retardation to the free movement of the cells has been observed near the boundary of the fluid and glass, all measurements have been made within the middle quarter of the observation chamber. The determinations recorded below are based upon duplicate or triplicate measurements of the diameter and from four to six or eight measurements of the velocity after it had become constant. When the mean square error was calculated upon the basis of forty-three duplicate measurements of the velocity and diameter of algal cells taken at random, it was found that for the former it was ± 0·0445 and for the latter ± 0·01588.

CONDITIONS OF EXPERIMENT.

In order to control the conditions under which the observations were made from day to day or hour to hour, as closely as possible, all measurements were made in the constant temperature room of the Physics Department in which the automatic self-recording balance is kept. In this way, viscosity changes due to temperature could be readily controlled to one-tenth of a degree. The constancy of the temperature is also an important factor in the elimination of convection currents due to temperature changes in the boundary layer of the liquid medium which is in contact with the glass walls of the observation chamber.

Since the cells could not be studied in distilled water, tap water was finally adopted as the suspending medium and was used in all cases. Injury to any of the cells was not observed at any time through its use. The water was drawn in quantity and allowed to come to room temperature before using. Its density was also carefully determined for a series of temperatures on either side of the average temperature at which it was to be used. In this, the tables of P. Chappius (*Bureau internat. des Poids et Mesures, Travaux et Memoires* XIII, 1907) were used as reference. It was found that the density of the tap water at the average temperature of the experiments (12·4° C.) was much nearer unity than that of distilled water under the same conditions. At 12·4° C., the actual density of the tap water used was found to be 0·99989 or equivalent to that of distilled water at 7·7° C. The density of distilled water for the indicated temperature is 0·99948. Since the records of actual cell density are given but to the third decimal place, the slight correction required through the use of tap water as a suspending medium has not been necessary.

EXPERIMENTAL RESULTS.

The formula of Stokes in which

$$V = \frac{2}{9}\,\frac{r^2(\rho - \rho_1)\,g}{\mu},$$

where V = velocity,
r = radius,
ρ = density of particle,
ρ_1 = density of suspended liquid,
μ = coefficient of viscosity of suspending medium,
g = gravity constant,

provides that with a knowledge of the rate of fall of a body of known radius in a liquid of known density and viscosity, then the density of the particle, within certain limits of size for particles of given density, can be calculated therefrom, since $\rho_1 - \rho = \frac{9u - V}{2r^2 g}$. All calculations are made with measurements in terms of C.G.S. units. For these studies the coefficient of viscosity of the medium was taken as for distilled water and the value for the temperature desired was obtained by interpolation by means of the formula of Everett(3) into the table of these values given by Kaye and Laby(4).

As an instance of the nature of the actual measurements performed, the average data obtained for large and small algal cells will be contrasted with similar measurements upon *Gonostomum* cysts. In view of the nature of the results given in more detail below, it would seem that the application of Stokes' formula to these studies is fully justified.

	Radius (cm.)^2	Rate of fall (cm./sec.)	Density $1 + (\rho - \rho_1)$
1. Alga	·000000052	·000134	1·146
2. "	·000000693	·000802	1·071
3. *Gonostomum* cysts	·00000218	·00252	1·065
4. " "	·00000580	·00561	1·054

From theoretical considerations Mr Fisher of the Statistical Department has found that for particles of the nature and density of those used in these experiments, the maximum particle diameter for which the formula might be expected to hold would be in the neighbourhood of 200μ. The largest particles used in these studies, however, were less than 50μ, thus leaving a wide margin of safety. With regard to the position, in this respect, of the particles of smaller size, it might be noted that for definite physical reasons the applicability of the formula becomes progressively less strained as the diameter of the particles decreases.

THE DENSITY OF UNICELLULAR GREEN ALGAE.

The distribution of about 120 random measurements are shown in Fig. 2 where the continuous black line represents the main axis passing through the vertex. The radius of the individual cell is plotted against the square root of its velocity, and it is seen that the position of any particles of given size, though varying widely in velocity, follows the main axis in a thoroughly consistent manner. The mean values calculated for the cells of different sizes, as grouped in Fig. 3, have been plotted as the broken line. In Fig. 3 the correlation between the mean density of the cells, as calculated from the mean radius and velocity, and their radius (curve *A*) is shown, together with the distribution curve for the sizes of the individuals. There is a consistent variation in density with size of the cells, that of the smaller being considerably greater than the medium or larger individuals.

The mean density is found to range from 1·07 in the case of the larger cells to nearly 1·15 in the case of the smaller.

The flat portion of the density curve is seen to correspond with that portion of the mean curve in Fig. 2 which most closely follows the axis. By analysing the relationship between the density and distribution curves it is seen that the larger part of the organisms studied are distributed through the flat portion of the former curve. Through this entire section from $r = 4$ to $r = 7$, the density only varies 0·006, and this portion of the curve represents 85 per cent. of the cells measured. Taking into account the distribution of the measurements with regard to size, the mean density is found to be 1·098, which is in good agreement with the value 1·090 found to fall upon the median of the distribution curve.

Without doubt the observed decrease in density, with increase in size of the algal cells, may be attributed to fundamental changes in the physical and chemical nature of the protoplasm as these organisms approach maturity. Thus, either the development of vacuolar structures in the protoplasm or the formation of oils, both of which are definitely known to occur, would serve to lighten the cell, as a whole, very materially. Under certain conditions where the cell contents are in an advanced stage of division and the cell wall is slightly distended with corresponding spaces within the cell wall unoccupied by protoplasm, the apparent density might be considerably decreased. In the present studies, however,

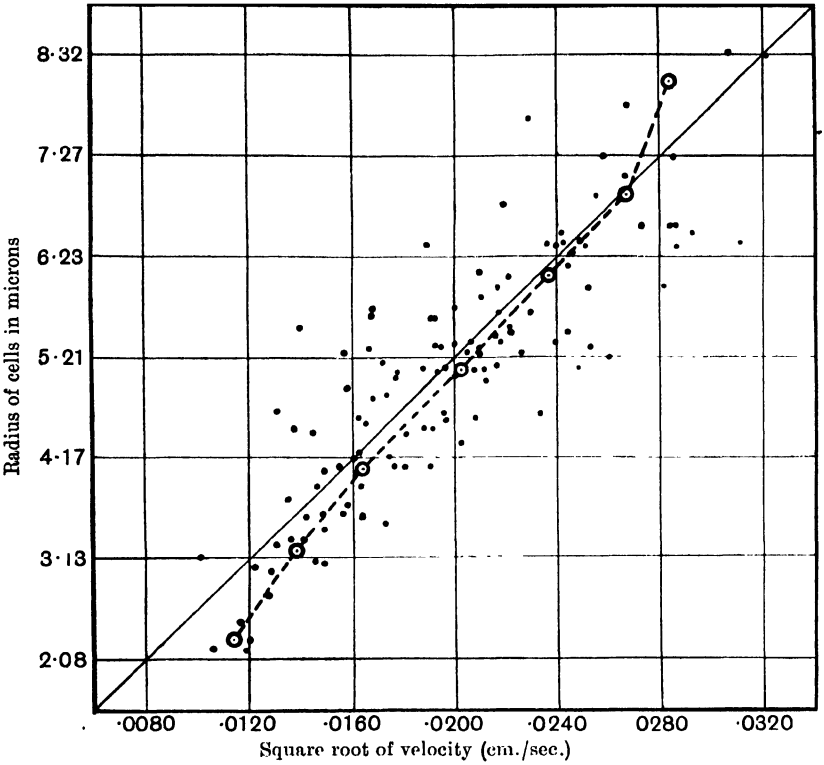

Fig. 2. A dot diagram with regression curve of average values for velocity and diameter.

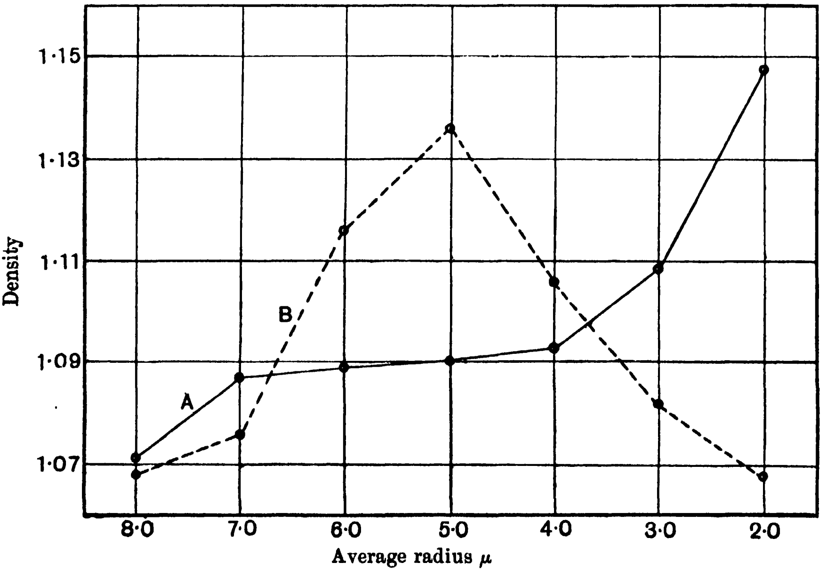

Fig. 3. The correlation (curve *A*) of density of algal cells with size, showing at (*B*) the distribution curve of the individuals measured.

measurements were not made upon cells where division was apparent in any way.

The fact that the density of the cells seemed to decrease so materially with age and development led to further investigation. Measurements were made upon a series of individuals from a culture which was three months old in which the green pigment had entirely disappeared and the cells had become greyish-yellow in colour. In this condition they were entirely inactive and possibly not a few of them were dead. Here the density was consistently low as compared with the young cells, and its variation with the size of the cell was not nearly so marked. The average value for the density was 1·057.

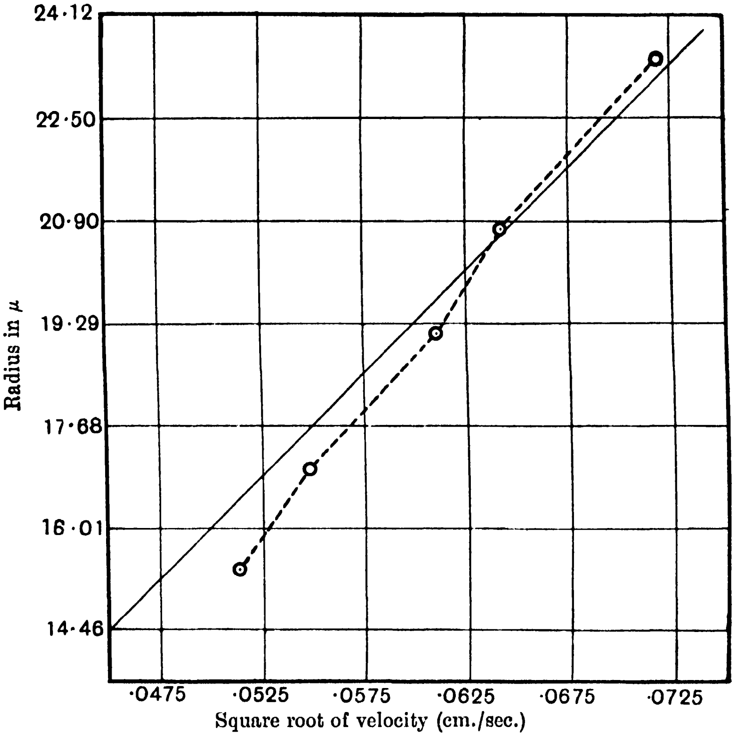

Fig. 4. The regression of velocity on diameter in the cysts of *Gonostomum* (sp.).

When grown upon a solid substratum, the central portion of the mass frequently loses its green colour and turns yellow, but in the margin of the culture, often the colour remains a deep green for some time. A considerable number of the green and yellow cells from such a culture were tested separately and the average density of the green cells for all sizes was 1·102, while that for the yellow cells was 1·060.

Further study was made upon cells from a flask culture upon solid substratum which had developed a brownish pigment. These were kindly furnished by Dr Bristol from one of her stock cultures. She regards the cells in this state as still vigorously active. The average density for all sizes in this group was found to be 1·094.

It would thus appear that in addition to the causes indicated above as possibly explaining the decrease in density of algal cells with increase in size and age, there may also be a point in the process of maturation, following which the actual condition of the protoplasm may become an important factor in reducing this value. This would hold particularly where the environment, for some cause or other, is inimical to the progress of normal growth.

THE DENSITY OF PROTOZOAN CELLS.

In studying the density of protozoan cells, because of the difficulty of paralysing or killing the active forms so that their bodies would remain unaltered both in form and composition, the cysts were employed. However, where the measurements upon the density of the cystic forms have been made under certain conditions, described below, an attempt has been made to correlate this value with that of the living active form.

DENSITY OF THE CYSTS OF *GONOSTOMUM* SP.

Gonostomum sp. is one of the larger types of soil protozoa, measuring from 80–120 μ in length, 20-30 μ in breadth and 5–8 μ in thickness. Thus, in general form, it is rather slender and sometimes quite thin. Its shape, however, like most protozoa will be determined to a considerable extent by the medium upon which it is grown and particularly the nature and extent of the food supply. This same type of organism is sometimes referred to in the literature as Gastrostylus. The average diameter of the cysts, measured in the course of these studies, was found to be 39·2 μ. They are usually brownish in colour, and appear quite dense with well defined margins bounded by fairly heavy, crenate walls.

In Fig. 4 the graph shows average radius values plotted against average velocities. All the points are not located since their distribution about the main axis was found to be quite similar in nature to that noted for the alga in Fig. 2. Particular attention is called to the proximity of the three middle points to the main axis which passes through the origin since, as will be pointed out later (distribution curve, Fig. 5), the larger part of the individuals measured fell in this region. The nature of the deviation of these points from a straight line independent of the main axis, suggests, as in the case of the algal cells, an inverse relation of density to size, though in this case the variation is much less marked.

The curve representing the actual variations in the density of the cysts is shown in Fig. 5 *A*. For convenience in comparing the density, as well as the nature of the variations between the *Gonostomum* cysts and algal cells, the curve for the latter is shown at *C*.

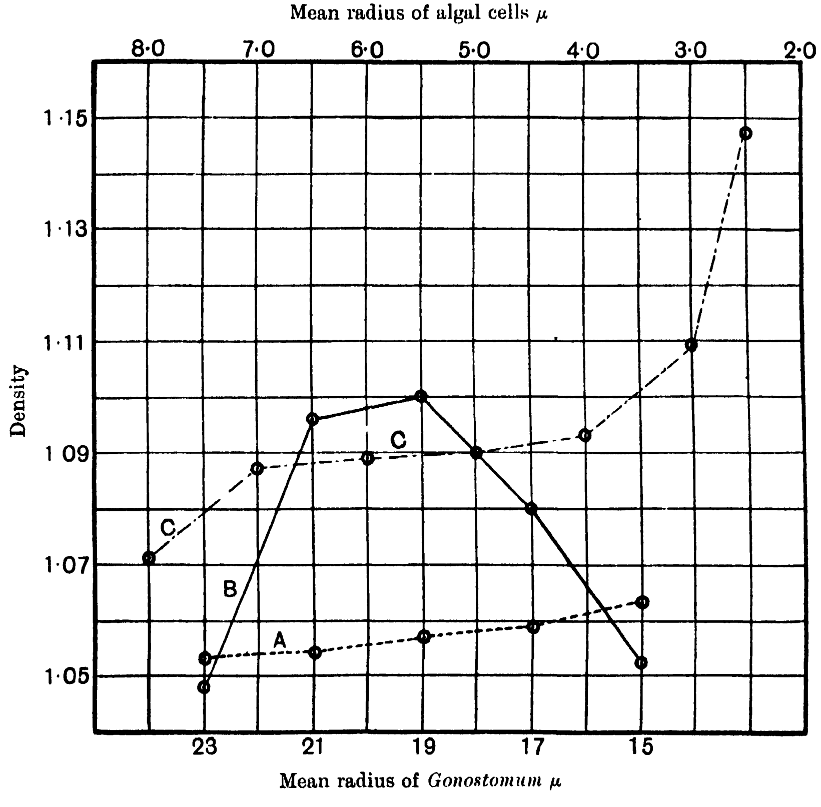

Fig. 5. The correlation of density of cysts (curve *A*) of *Gonostomum* (sp.) with diameter; (*B*) being the distribution curve of numbers measured, and (*C*) the comparative correlation curve for algae (see Fig. 2).

It is seen that the total variation in density in the case of the cysts is strikingly smaller than that for the algae. Likewise, the variation, though slight, is much more constant in the case of the cysts throughout the series. From this it would appear possible that an age factor in its relation to the maturation of the individual cyst and consequently its size and density, may be definitely correlated with this tendency. This will be referred to again where, in the case of the young and old cysts of a certain species of *Colpoda*, it is most marked.

The distribution curve for the *Gonostomum* cysts measured is also shown in Fig. 5 *B*. Thus the three middle points whose total variation in density is but 0·005 represents 91 per cent. of the individuals examined. The average density calculated for the entire population measured is 1·057; this coincides exactly with the median value found under the apex of the distribution curve. Thus, in terms of comparative average values, the density of the algal cells is 1·089 while that of the protozoan cysts is 1·057 or about 35 per cent. less.

THE DENSITY OF CYSTS OF *COLPODA* SP. AND OF *HARTMANELLA HYALINA*.

In studying the density of the cysts of *Colpoda* sp. while no particular difference was expected from those of *Gonostomum* when mature cysts were used in both cases, the variation in size of the former with age made it desirable to investigate them particularly from this standpoint. The *Colpoda* sp. used was an unusually large type, especially in the young cultures when the food material was particularly abundant.

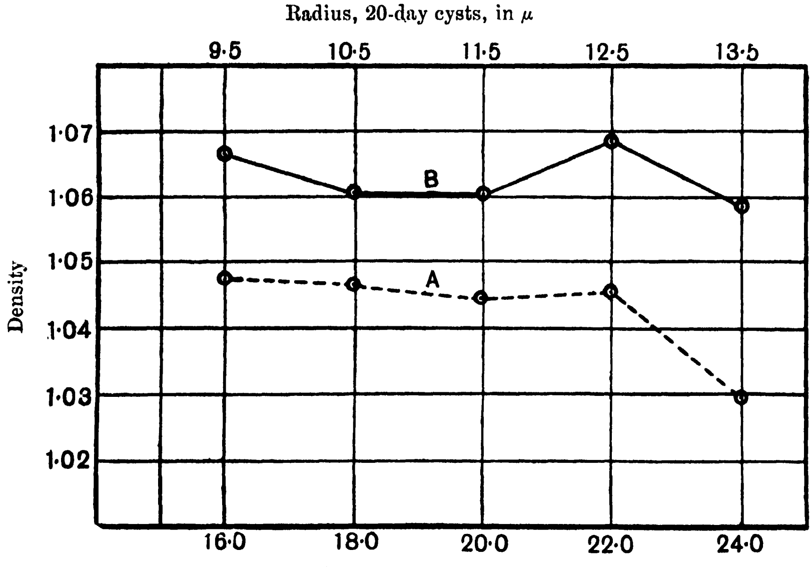

Fig. 6. The correlation of density (*A* 4-day, *B* 20-day) with the degree of maturation of cysts of *Colpoda* (sp.).

Another point which has proved of considerable interest in the case of the young cysts is the quickness with which excystation occurs. This was emphasised upon washing a set off the plates, in order to transfer them to the tap water in which they were to be studied; upon allowing them to stand for short periods to settle between successive washings it was found that in about five hours practically all had excysted. This process did not occur in so short a time in any case with more mature cysts.

Four-day and 20-day cultures were selected for comparison. The average diameter of all the cysts measured from the former was 40·1 μ and from the latter 25·12 μ. The 4-day cysts were washed and immediately suspended in tap water and the measurements usually completed within an hour after their removal from the plate. The average individual density for each group was 1·042 and 1·061 respectively. The fluctuations in density with size as well as the relative density of the various sizes between the two groups is shown in the graph of Fig. 6.

It is evident from the figure that with the progress of maturation, the density has increased very materially.

DRY WEIGHT OF CYSTS.

In order to analyse further the nature of this loss in weight of the cysts during maturation, an attempt has been made, upon the basis of their observed density, to apply the formula of Hehner and Richmond to the estimation of the actual dry matter which the cysts contain. This formula

$$T = 0{\cdot}2625 \frac{G}{D} + 1{\cdot}2F,$$

where T represents total solids, D the specific gravity, $G = D - 1$ and $F =$ the percentage of fat, has been developed particularly for the estimation of the total solids in milk when the density of the whole liquid and the fat content are known. While the involved assumption that the difference in the specific gravity of the milk serum and water is directly proportional to the percentage of solids in solution is not always strictly correct, Wiley (6) states that the error arising from this source, even in extreme cases, is never more than 0·5 per cent.

Neglecting the small amount of fats and waxes which the cysts probably contain, it is found that, by the application of the formula, the calculated dry matter in the young cysts amounts to 10·6 per cent. while that for the mature cysts is 15·1. Collecting together into a single table the data that have been obtained upon the two sets of cysts by the above method we have:

	Cysts of *Colpoda* sp.	
	Young (4-day)	Mature (20-day)
Average diameter	40·1	25·12
Average density	1·042	1·061
Weight (mg.) of individual cyst	·00003518	·00000881
Percentage dry matter	10·6	15·1
Dry matter (mg.) per cyst	·00000373	·00000133
Water (mg.) per cyst	·000003145	·00000748
Total loss in dry matter		·00000240
Total loss in water		·00002397
Water percentage of total loss		90·9
Dry matter percentage of total loss		9·1

From this, it would appear that while the cysts lose a considerable portion of their dry matter (64 per cent.) during maturation, the loss of water (76·2) is considerably greater through the same period. With regard to the relative loss of the two constituents, it is found that 90·9 per cent. of the total loss in weight is due to water and 9·1 per cent. to dry matter.

If the value 6·8 per cent. of neutral fat for the protoplasm of Plasmodium, as found by Lepeschkin (5), be tentatively adopted in these calculations as the content of analogous materials in these cystic bodies, it will only increase the calculated dry matter in either case by a little over 1 per cent. The water content of the protoplasm of Plasmodium was given as 17·4 per cent.

Calculating the dry matter for the algal cells in the same manner upon the basis of average density, it will be found to be 23·43 per cent. while that for the *Gonostomum* cysts is 14·15 per cent.

THE DENSITY OF ACTIVE PROTOZOA.

With regard to the density of the active organisms, due to the difficulty of killing or paralysing them as well as measuring and calculating the volume of their irregular bodies, as was pointed out above, the nearest approach that has been made to such a value has been in the use of the young cysts discussed above. In a number of cases while their measurement was in progress the protoplasm of these cysts began to rotate, an activity that is usually found to precede excystment. In some cases shortly after the measurement of the cyst was completed it divided into two or four young organisms which after some difficulty finally became separated and swam away as free individuals. Thus instead of trying to measure irregular and active forms, which in no way now known can be caused to fall inertly and uniformly through the suspending liquid, it is much simpler to measure the cystic form immediately before excystation occurs. Since the bodies of the young animals constitute the entire mass of the mother cyst it would seem that the density of the latter would represent fairly closely the density of the daughter cells at the time of their separation.

Since there is no consistent variation in the densities of those cells which give evidence of excysting or did excyst shortly after their observation was completed as compared to the other cells of the same age, it is thought that the average density (1·042) presented above for the entire group of 4-day cysts may represent fairly closely the density of the active organisms excysting therefrom, directly the process is completed. While there may be a slight difference in density in young and freshly excysted animals as compared to older individuals, it is thought that this difference will not be found to be great. This, however, is a matter which cannot be discussed upon the basis of the data available at the present time.

THE DENSITY OF *HARTMANELLA HYALINA* CYSTS.

The measurements so far carried out upon the density of *Hartmanella* cysts have not been entirely satisfactory. Therefore the average density of 1·084 as calculated from the results of some fifteen measurements will be presented as tentative until the value can be worked out upon the basis of larger numbers. With regard to the relation of size to density, the latter increases directly with size, the range being from 1·064 to 1·104.

These studies were carried out in the Department of Protozoology at Rothamsted, and it is a pleasure to thank Mr Cutler for his critical interest during the progress of the work and during the preparation of the manuscript.

SUMMARY.

1. By measuring the rate of fall of certain unicellular organisms of known diameter under appropriate conditions, their density has been calculated by the formula of Stokes.

2. According to this method the average density of algal cells studied is 1·098 and that for the cysts of *Gonostomum* sp. 1·057.

3. The density of the algal cells was found to vary greatly between the larger and smaller sizes, while that for cells intermediate was fairly constant. In the case of protozoan cysts the total variations in average density was much less marked.

4. It was found that during maturation, the cysts of a certain species of *Colpoda* decreased to one fourth their original volume, while their average density increased from 1·04 to 1·06.

5. Since many of the 4-day cysts of *Colpoda* sp. excysted during or shortly after the measurement of their density, the average density, 1·042, for this group is thought to represent quite accurately the density of the young animals in this early stage.

6. By the application of the formula of Hehner and Richmond to the density values so obtained, a tentative value has been derived for the actual dry matter of the cells studied.

7. It has been found by calculation upon this basis that the dry matter of the young cysts (4-day) of *Colpoda* sp. amounts to 10·6 per cent. while at the later stage (20-day) it is 15·1 per cent.

8. It appears that 64·3 per cent. of the total dry matter of the cyst and 76·2 per cent. of the water content are lost during maturation. With regard to the total percentage of loss in terms of either constituent, it has been found that water represents 90·9 per cent. and dry matter 9·1 per cent. of the total loss respectively.

REFERENCES.

(1) Bristol, B. Muriel and Page, H. J. (1923). A Critical enquiry into the alleged fixation of Nitrogen by green plants. *Ann. App. Biol.* x, 378–408.

(2) Cutler, D. W. (1919). Observations on Soil Protozoa. *Journ. Agric. Sci.* ix, 430–443.

(3) Everett, J. D. (1900). On a Central-difference Interpolation formula. (Read Sept. 10.) *Brit. Assoc. Rep.* pp. 648–650.

(4) Kaye, G. W. C. and Laby, T. H. (1916). Physical and Chemical Constants and some Mathematical Functions. Longmans Green & Co., pp. 153. London; New York.

(5) Lepeschkin, W. W. (1923). Über die chemische Zusammensetzung des Protoplasmas des Plasmodiums. *Ber. deut. Bot. Geselb.* xli, 179–187.

(6) Wiley, H. W. (1914). Principles and Practices of Agricultural Analysis. *Agricultural Products,* iii, 846.

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THE MULBERRY "BLIGHT" IN BRITAIN

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(With Plate II.)

A DISEASE of mulberry trees, recorded for this country in the present note, first came under the writer's notice as long ago as 1914. From material obtained in 1915 a white bacterium was isolated which, by inoculations made in the spring of the following year, was shown to be the organism causing the characteristic lesions. Pressure of other work at the time prevented a study of the various physiological reactions necessary for the complete identification of the organism but from the general symptoms of the disease it was suspected that the organism was *Bacterium mori* which has been shown by Dr Erwin F. Smith to be the cause of mulberry "blight" in France and in America. The organism was kept under culture and, as the occurrence in Britain of a pathogenic mulberry bacterium remained unrecorded, it was considered desirable that its identity should be determined. Recently the necessary tests have been carried out and as they confirm the previous impression that the organism is *Bacterium mori* a brief description of "Mulberry Blight" as occurring in this country may be of interest.

Early in July, 1914, diseased mulberry shoots were sent from Bognor, Sussex, to Wye College for examination. The shoots showed a "die-back" apparently caused by *Fusarium lateritium*, this fungus being present on cankered areas at the base of the diseased portions of the shoots[2]. Some of the leaves however showed dark spots resembling those described as produced by *Bacterium mori*, but as the main trouble appeared to be that caused by the fungus the leaf-spot was not further investigated at that time.

In August, 1915, mulberry leaves showing similar black lesions were received from a nursery in Kent. Some of the leaves had only a few small scattered spots but others were more seriously affected, the spots being more numerous and often reaching a diameter of 2 mm. individually, while coalescing spots were not infrequent. The spots themselves were angular in outline, often distinctly so, being delimited by the smaller veins which formed borders to the spots. Each black spot on these badly diseased leaves, as seen from above, was surrounded by a zone of yellowish green tissue. This zone was about 1 mm. across but gradually merged into the general green of the leaf; when several spots were in close proximity the zones became confluent, forming irregular yellowish areas containing the black spots. This pale zone was not noticeable on the lighter lower surface but it was easily seen by holding a leaf up to a window when, by transmitted light, it had a water-soaked appearance. The black spots were invariably found to be crowded with bacteria.

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[1] The inoculation experiments and most of the physiological tests were carried out at the South-Eastern Agricultural College, Wye.

[2] See *Gard. Chron.* Aug. 26, 1916, p. 95.

In the nursery the disease was chiefly on young trees which had been removed from the parent "stool" the previous year after having been layered for some five years. A close examination of these young trees showed that not only were the leaves infected but that black areas were to be found on the bark of the young shoots also. The disease had been noticed on these young trees during the previous season about three months after they were planted out. The stools (about 60 years old) from which the layered shoots had been taken also bore a number of diseased leaves but the spotting was not nearly so pronounced as on the young trees planted out. A few diseased leaves were also found on a number of well-established trees (about 20 years old) which were growing in close proximity to the young trees.

From the nature of the case it appears probable that the outbreak originated in the stool-bed, that the shoots when removed from the stools were already infected to some extent, and that the conditions obtaining when the trees were planted out favoured the spread of the disease.

The first isolation plates, prepared in 1915 from infected Kent material, produced bright yellow colonies only, but shortly afterwards specimens from Bognor yielded plates consisting of white colonies with a few yellow ones. In the following year three young trees with spotted leaves were received from the nursery referred to above and isolation plates gave rise in this instance to white colonies only, similar to those obtained from the Sussex specimens.

In 1916 a young healthy mulberry tree[1] growing in a pot in the greenhouse was inoculated with the white and with the yellow organisms.

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[1] This tree was kindly presented for inoculation experiments by Mr A. E. Bunyard of Maidstone.

Drops of condensation water containing the particular organism were taken from cultures 24 hours old and placed on selected leaves. Three leaves were inoculated on the upper surface and three on the lower surface without wounding, while three others were punctured at the inoculated spots. On each leaf four drops of the inoculum were so placed as to form, roughly, the corners of a square on one side of the midrib; where a leaf was wounded control punctures (not inoculated) were made on the other side of the midrib of the same leaf.

The yellow organism produced no infection whatever. The white organism on the other hand readily produced black spots at all the inoculated punctures and in a few instances black spots appeared at the inoculated spots not punctured. On the sixth day after inoculation the spots successfully infected were seen as small water-soaked areas best observed by transmitted light; later these became black spots about 1 mm. in diameter surrounded by a yellowish zone. The black spots remained about the same size but the yellowing extended. One of these leaves was removed about five weeks after inoculation and is shown in Pl. II, fig. 2; the spots were found to be swarming with bacteria and from one of the spots the organism was re-isolated. The other infected leaves turned yellow and fell comparatively early in the season.

The two organisms were used in another experiment in which the leaves of certain branches were sprayed with suspensions of the bacteria, some of the leaves being punctured. Again the yellow organism produced no blackening and the leaves inoculated with it showed no injury apart from the actual puncturing. The white organism produced, as before, the characteristic spots on all the punctured leaves but not on those uninjured, a fact suggesting that the bacterium requires a wound through which it can gain an entrance, although in the previous experiment a few black lesions appeared at spots inoculated without puncturing. The eight infected leaves eventually turned yellow and fell prematurely.

Control branches were sprayed with sterile water and some of the leaves punctured; of some thirty punctures on these control branches blackening appeared at six, but later than on the leaves primarily inoculated and they probably became infected indirectly from the other spots.

A third inoculation experiment was carried out the same summer using the organism re-isolated from one of the leaves infected in the first experiment. Inoculated punctures again gave rise to the typical lesions with a premature fall of the infected leaves.

The inoculations were continued in 1919 on a tree (in a pot) in the open, using a sub-culture of the original white organism. Again, inoculated punctures readily became infected and from one of the spots the bacterium was re-isolated as shown by successful inoculations carried out in 1921 and 1922 using this re-isolated line.

In these later experiments it was found that inoculated punctures made on the internodes of the shoots gave rise to lesions resulting in some cases in the death of the portions terminal to the punctures. Thus in each of seven out of ten inoculated shoots the whole of the internode on which the inoculation was made became blackened and that portion of the shoot terminal to the wound died, while on each of the other three there was a black longitudinal lesion 3 to 10 mm. in length.

The experiments show clearly that the black lesions on the leaves and shoots are caused by the white bacterium used in the inoculations; the yellow organism is obviously a saprophyte in the spots and after the earlier inoculation experiments it was not studied further. That a yellow organism is frequently found associated with *Bacterium mori* has been commented on by Smith (1). Italian workers have attributed this or a similar disease to a yellow bacillus.

The morphology and physiology of the white organism conform closely with the descriptions of *Bacterium mori* given by Erwin F. Smith (3) who was the first to describe it fully enough for identification, and by Doidge (1) who, in a paper recording the occurrence of mulberry "blight" in South Africa, gives a history of the disease and a description of the organism. As Dr Doidge's article appeared in this *Journal* it is unnecessary to deal with these points in detail, especially as they are also easily accessible in a recent book by Dr Erwin Smith (6). Its growth on nutrient agar, nutrient gelatine and potato plugs is as described by the authors mentioned, as is also its behaviour in nutrient broth, Uschinsky's solution, milk and litmus milk; it does not liquefy gelatine or solidified blood serum. It is strongly aerobic as shown by the fact that, in the writer's experiments, in fermentation tubes containing peptone solution with either glucose, glycerine, lactose, maltose, mannite or saccharose the liquid in the closed limb remained clear for about three months; in fact turbidity did not extend into the closed limb until evaporation had proceeded so far that a bubble of air appeared in that limb.

In broth containing 6 per cent. sodium chloride the organism made a little stringy growth; an examination of the resulting sediment showed it to consist of filamentous involution forms. The filaments were so intertwined that they were not easily measured but in some cases where they were floating free they could be measured and were found to be frequently over 100μ in length; the longest measured was 180μ long. Intercalary or, more often, terminal on the filaments were fusiform or spherical bodies, the largest seen being 8μ in diameter. Both Smith and Doidge found involution forms in broth containing 6 per cent. sodium chloride but did not describe them.

A little stringy growth was also obtained in bouillon containing 6·5 per cent. sodium chloride but there was no growth with 7 per cent. Doidge records feeble growth in broth containing 6·5–7·5 per cent.

There was no growth in Cohn's solution—Doidge records "no growth" and Smith "no growth or only very slight." No indol could be detected in peptone water cultures and there was no reduction of nitrate to nitrite in nutrient broth containing 0·5 per cent. potassium nitrate.

The organism is very sensitive to sunlight. In the writer's experiments plates were poured and then exposed (bottom upwards on ice) to the midday sun in November, one half of each plate being shaded with black paper. The shaded half in each case gave rise to many colonies; on the unshaded half exposure for 30 minutes killed 90 per cent. of the organisms on one plate and all of them on another; on plates exposed for one hour no colonies appeared except on the shaded half.

It is resistant to desiccation; coverglass smears were not killed by several days' drying at room temperature; on one occasion a coverglass smear was found to be viable after drying for 30 days.

The thermal death point is round about 51° C.; Smith gives it as about 51·5° C. while Doidge found 50° C. to be sufficient. The writer found that temperatures of 48° to 50° for 10 minutes retarded the appearance of turbidity in inoculated tubes while a temperature of 51° C. resulted in no growth whatever.

As in Doidge's and in Smith's later trials the rods were found to have several polar flagella; by staining with Muir's Pitfield method and particularly by Moore's modification of Loeffler's one to six flagella were demonstrated. The method recommended by Plimmer and Paine[1] (2) also proved a quick and easy method of staining the flagella of this organism.

Control. There appears to be no satisfactory method known of controlling this disease on old trees especially if the trouble is of old standing, and in the literature consulted the case of nursery stocks is not discussed. It is obvious however that healthy "stools" should be used for propagation and seriously affected stools should be destroyed by burning. If a stool is slightly affected diseased layers should be cut out and burnt;

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[1] This test was carried out by Mr R. V. Harris, working in the Pathological Laboratory at the East Malling Research Station.

on no account should layers showing black lesions on the leaves or stems be planted up. Young trees should not be bedded out in the neighbourhood of established mulberry trees, particularly if the latter are known or suspected to have the disease.

SUMMARY.

1. The occurrence of bacterial Mulberry "Blight" in Sussex and Kent is here recorded.

2. It has been found on well-established trees, on "stools," and on young trees during the first and second years after removal from the stools.

3. The bacterium causing the disease has been isolated from lesions on the leaves and this has been found to conform to descriptions of *Bacterium mori* Boyer and Lambert.

REFERENCES.

(1) Doidge, E. M. (1915). The South African Mulberry Blight, *Bacterium mori* (Boy. and Lamb.) Smith. *Ann. App. Biol.* II, 113–124.

(2) Plimmer, H. G. and Paine, S. G. (1921). A New Method for the Staining of Bacterial Flagella. *Journ. Path. and Bact.* XXIV, 286–288.

(3) Smith, E. F. (1910). Bacterial Blight of Mulberry. *Science N.S.* XXXI, 792–794.

(4) —— (1912). Bacterial Mulberry Blight. *Phytopathology,* II, No. 4.

(5) —— (1914). Identity of the American and French Mulberry Blight. *Ibid.* IV, No. 1, 34.

(6) —— (1920). *An Introduction to Bacterial Diseases of Plants.* Philadelphia and London.

EXPLANATION OF PLATE II

Fig. 1. Branch of naturally infected mulberry tree; in centre a healthy twig, on right and left twigs with diseased leaves which are generally paler than normal leaves but have black bacterial spots.

Fig. 2. Leaf inoculated from a pure culture of *Bacterium mori*; on the right inoculated punctures have given rise to black spots with a pale zone round each; on the left of the midrib are four control punctures.

Fig. 3. Leaf inoculated by spraying with a suspension of the organism; black spots have arisen only at the punctures.

(*Received March* 18*th,* 1924.)

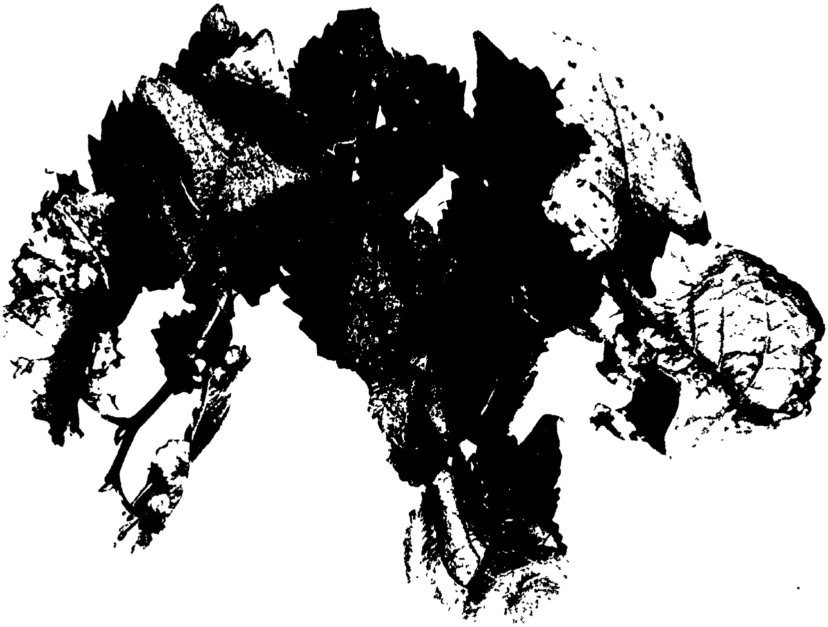

Fig. 1

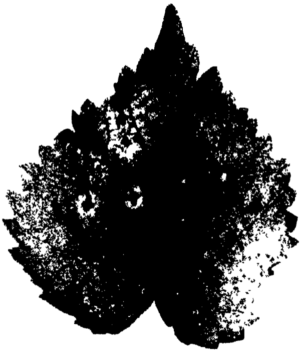

Fig. 2

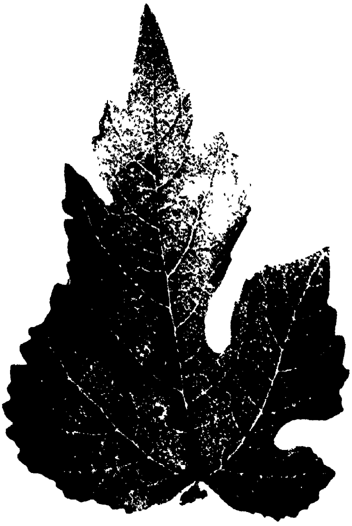

Fig. 3

WORMALD.—The Mulberry "Blight" in Britain. (pp. 169—174.)

ON *CHLOROPS TAENIOPUS* MEIG. (THE GOUT FLY OF BARLEY)

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(*From the Entomological Dept., Rothamsted Experimental Station.*)

(With 4 Text-figures and Plates III and IV.)

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INTRODUCTION.

The following paper deals with observations and experiments upon the life-history of *Chlorops taeniopus*; the work has been carried out at Rothamsted Experimental Station during the past two years under the direction of Dr A. D. Imms. Wherever possible, I have tried to put field observations upon a strictly numerical basis, and the advantages of this method have been very great. It is impossible to publish the mass of data accumulated, but in most cases I have indicated in the text the type and the extent of the data upon which my conclusions are based. Several interesting lines of work, particularly in regard to the habits and tropisms of the imagines, have scarcely been touched upon: attention has been concentrated mainly upon those aspects of the problem which seemed to offer the greatest probability of indicating control measures.

I have not given a complete list of the literature relating to *Chlorops taeniopus* because the position of the whole question up to 1870 is admirably summarised by Nowicki (3). He refers to about 40 different papers (some of which I have been unable to obtain) and gives many new observations of his own and of a farmer who collaborated with him. Since 1871 practically no important new work has been published. The best account of the pest in England is that by Ormerod (4). Fulmek's paper (2) contains a few new observations mainly with regard to the winter generation.

I wish here to express my sincere thanks to Dr A. D. Imms for much valuable advice during the progress of this investigation and for his constant willingness to discuss difficult points which have arisen. This opportunity is also taken to acknowledge the valuable assistance given in various ways by Dr W. E. Brenchley, Miss W. A. Mackenzie and Messrs J. E. Collin, S. J. K. Eames, R. A. Fisher, H. V. Garner, E. Somerfield and W. Young.

LIFE-HISTORY.

Chlorops taeniopus has two generations per year, a winter and a summer, the former covering much the longer period of the two. A typical life cycle is as follows. The flies emerge from their winter host (couch grass[1]) towards the end of May and lay their eggs on the leaves of the shoots of spring barley. After about eight days the eggs hatch and the young first instar larvae make their way into the centre of the shoot to the young ear. Starting from the apex of this they eat their way down one side of the ear and down the internode below it, gnawing a dark brown food groove. When mature the larva turns round, still within the shoot, until its head is directed upwards. It then ascends a short distance and pupates within the host shoot. Pupation occurs during July and the pupal period is about 36 days. The majority of the flies emerge during August but a few may be much later. These flies lay their eggs on couch grass and the larvae pass the whole of the winter head downwards in the grass shoots. Towards the end of February in the following year the mature larvae turn round within the shoots until the head is directed upwards and pupate, within the host shoots, during the early part of March. The pupal period is slightly over 60 days and the flies emerge during the end of May and the beginning of June.

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[1] *Agropyrum repens.*

The different stages of the life cycle may now be examined in more detail.

The eggs are laid singly on the leaves of the host plant—usually on the upper side of the lamina fairly close to the ligule, but occasionally far from the ligule, near the tip of the leaf. They are also fairly frequently laid on the under surface of the leaf; and rarely, on the sheathing part of the leaf, altogether below the ligule.

The following figures give the relative numbers of eggs found on the upper surface of the lamina, its lower surface, and on the leaf sheath. The figures are taken from certain counts of eggs on spring barley in 1923 which will be referred to later, and as they represent the results of an examination of over 3000 shoots they are probably fairly exact:

Upper side of lamina	Lower side of lamina	Sheathing part of leaf
360 (65·1 %)	161 (29·1 %)	32 (5·8 %)

Out of a total of 490 shoots bearing eggs only 53 (10·8 per cent.) bore two eggs and only 5 (1 per cent.) bore 3 eggs, all the remainder bearing only one egg per shoot, no shoot being found bearing more than three eggs. Out of a total of 508 leaf laminae bearing eggs only 11 (2·14 per cent.) bore two eggs, and only one bore three eggs.

The eggs always lie between two adjacent longitudinal ridges of the leaf surface. One end of the egg, the anterior, is slightly dorso-ventrally flattened and this end is always directed towards the apex of the leaf. The egg is fastened down to the leaf surface by a small amount of a colourless cementing material. Upon hatching the anterior end of the egg splits into dorsal and ventral halves. The appearance of the egg after hatching is extremely variable; the two halves of the split anterior end may close together so perfectly after hatching that only by microscopic examination can one be certain whether or not it has hatched, as the egg shell itself is quite opaque both before and after hatching and does not change its white colour. Frequently, however, the two halves of the anterior end of the egg remain separate after hatching, or the empty egg may crumple up considerably. These differences after hatching are probably due to differences in atmospheric humidity.

The eggs do not fall from the leaves immediately after hatching but remain for some time, being probably eventually washed off by the rain. Possibly the growth of the leaf tissue after the egg is laid gradually cracks and loosens the cement which fastens the egg to the leaf.

During the latter half of August 1922, ten observations were made on the period of incubation of eggs laid at this time; each of the ten observations gave either 8 or 9 days. No careful observations have been made on the period of incubation of eggs laid in early summer (*i.e.* those laid by flies emerging from the winter generation) but it is probably little, if any, different from the period given above. The incubation period probably varies slightly with low or high temperature.

Larval Stages.

The morphology of the larval stages has been described elsewhere (1). The actual hatching of the egg has unfortunately never been observed so that the actual penetration of the first instar larva has not been seen. It is almost certain that the eggs normally hatch some time during the night —probably in the early morning hours, and although various conditions of humidity, etc., have been tried I have not succeeded in the laboratory in getting eggs to hatch during the day time.

It also seems probable that the first instar larvae must penetrate into the plant during the night. On several occasions small pieces of leaves bearing eggs nearly due to hatch have been left in glass tubes overnight. The eggs have hatched and the first instar larvae easily found in the morning. Not one of these larvae, however, has ever penetrated into a plant upon which it was placed in the day time.

After numerous dissections of barley shoots containing first and second instars of *Chlorops* the mode of penetration of the larva seems to me to be as follows. The larva upon emerging from the egg has its head directed towards the apex of the leaf. It must therefore turn round before entering the shoot. Supposing the egg to have been on the upper surface of the leaf the larva makes its way down to the ligule, crawls round between the ligule and the lamina until it comes to the slit between the overlapping edges of the sheathing portion of the leaf, and through this slit it enters the shoot. It then usually works inwards and downwards in a spiral manner through the slits between the edges of successive sheathing leaves until it reaches the centre of the shoot. Instances have been seen where the larva has bored directly through one or more of the inner sheathing leaves on its way to the centre of the shoot. Nowicki (3) suggested that the larva might possibly penetrate into the plant by boring through the ligule, but I think this very improbable. Although, as stated above, the first instar larva does occasionally bore through the tender inner sheathing leaves, it is not, I imagine, sufficiently strong to bore through the relatively tough and membranous ligule of one of the outer leaves. The larvae from eggs laid on the lower side of the lamina, or on the sheathing part of the leaf, similarly enter the shoot between the approximated edges of the sheathing leaves.

The first larval instar is apneustic (1) and cannot therefore be expected to have a long existence. As I have not been able to observe the actual penetration of a first instar larva into a shoot I cannot state definitely the duration of this phase, but it is almost certainly less than 48 hours. First instar larvae which have hatched out one night have been found to have migrated far into the shoot when this was dissected next morning. The first instar larva is, in fact, essentially a migratory phase. It undoubtedly feeds to some extent, as green chlorophyll coloured matter can be found in its gut, and also it grows somewhat before moulting into the second instar. I have, however, never been able to find any marks due to the feeding of the first instar, except in the comparatively rare cases where it has bored through one of the inner sheathing leaves. The primarily migratory nature of the first larval stage is a common feature among parasitic Cyclorrhaphous larvae, or indeed among animal parasites in general.

In a typical case of infection by the summer generation the first instar larva reaches the apex of the tender young ear before changing into the second instar; and in a typical case of winter attack it attains almost to the base of the shoot. This point as regards the summer attack will be discussed in detail later. Deformation of the plant (see below) is not apparent until the larva reaches the second instar. This does not mean that deformation may not be initiated by the first instar, because the deformation would necessarily take some time to become apparent after the impulse to deformation had been given, and the life of the first instar is only of short duration.

The feeding habits of the second and third larval instars will be found discussed below in connection with their effect on the plant, and to avoid repetition a few general points only are considered here. The first and second instar larvae are subject to a definite tropism causing the first instar to migrate towards the base of the leaf upon which it hatches, and causing both first and second instar larvae to move downwards when within the shoot. The tropism is probably primarily a food one which has become an ingrained larval habit. In the majority of cases this habit is beneficial and leads the larva to its food, but in certain rather rare conditions, which will be discussed in connection with the effect of manures on infestation, the habit acts against the larva and destroys it by leading it away from suitable food. That the tropism is not a gravitational one is shown by the fact that larvae hatching out from the egg will enter the shoot upon which they hatch even if it is placed in a horizontal position[1], and if the tropism is not gravitational for the first instar it is probably not so for the second and third instars. What little evidence I have upon these points is quite fortuitous and derived from data obtained to determine entirely different questions.

In the winter generation the third instar larva does not usually move downwards at all, in the summer generation it usually does so (see below, Effect on the Plant). In both cases the mature larva, which is head downwards during the greater part of its life, turns round a short time prior to pupation so that its head is directed upwards. This is almost certainly not an effect of gravity because if mature larvae are removed from their host plant and placed upon moist filter paper in tubes, they will pupate horizontally, head up, or head down, and normal healthy flies will emerge. It cannot be due to a suddenly acquired positive heliotropism because in the great majority of cases no light can penetrate to the larva. It is undoubtedly connected in some way with a change in the physiological state of the larva when it becomes fully fed. In all cases after turning round the larva migrates upwards a short distance before pupating and it may be a pressure stimulus which causes the larva to seek a relatively spacious site for its pupation. Instances have been seen of puparia which failed to develop because pupation occurred in too restricted a space and the puparium was consequently flattened. Apart from the probable necessity of finding space for pupation, if a larva pupated head downwards the fly, when it emerged, would very probably fail to escape from the shoot.

In the case of the summer attack (see below) pupation may occur in the food groove on the ear bearing internode, on the ear, or above the ear. It was thought that the distance of upward migration of the larvae might be constant, within small limits, for all larvae and that the position of pupation with regard to the ear depended upon the dimensions of the shoot. This is not so, as measurements of the length of migration were made on 50 shoots and were found to vary from ¾ in. to 6¼ ins. The distance of upward migration of the winter larvae was not measured, but the attacked shoots themselves are sometimes less than 1 in. in length and the migration therefore necessarily restricted. Larvae never leave the host plant to pupate either in the winter or summer generation.

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[1] Unfortunately the experiment of inverting the shoot was not carried out. The horizontal position does not give quite definite proof that this tropism is not to some extent gravitational.

EFFECT ON THE PLANT.

(Plates III and IV.)

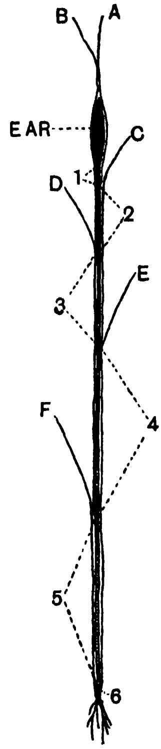
The general effect of an attack of either the winter or summer generation of gout fly is to produce stunting and thickening of the attacked shoot with considerable increase in the breadth, and moderate increase in the thickness of the leaves. The deformation produced by the winter attack and by the summer attack have usually been described as entirely different in appearance and nature. While this is incorrect if all forms of the damage are considered one can nevertheless distinguish quite sharply the typical form of winter damage and the typical form of summer damage, and this is the most convenient way of describing the various intermediate forms. For the sake of convenience barley is considered as the host for both winter and summer generations, although couch grass is a far more usual host for the winter generation.

Typical winter form (on winter barley). (Plate III, fig. 1.)

Attacked shoots are short and very thick, with broad, short and somewhat thickened leaves having a healthy green colour. No stem is recognisable, being so extremely stunted that the leaf sheaths all appear to arise at practically one point at ground level. The thickness of the shoot is due to the thickness and breadth of the separate leaves which surround each other. The edges of the leaf laminae are usually considerably crinkled and the leaf veins are usually wavy instead of straight. The laminae of the more central leaves are curled in a spiral manner; the outer ones less so or not at all. The tips of the leaves are frequently dead and frayed out. This condition of the plant makes its appearance about the beginning of December (earlier or later according to the date of oviposition upon it) and is maintained without change or growth all through the winter until towards the beginning of March, when the larvae commence to pupate. As soon as a larva pupates the attacked shoot commences to wither and die, and decay soon sets in. It seems probable that the feeding larva secretes some substance (probably from its salivary glands) which maintains the attacked shoot in the green and sappy condition necessary for the nutrition of the larva.

Typical summer form (on spring barley). (Plate IV, figs. 1 and 2.)

While the great majority of attacked winter shoots are of the type described above, the type of distortion produced by the summer attack varies very greatly and depends entirely upon the stage of growth of the shoot when attacked, while the degree of distortion produced depends upon the rapidity with which growth is progressing at the time of the attack. The typical form will show a stem of two or three internodes, and then a thick upper region which consists of the ear completely enclosed in its sheathing leaf from which it never escapes. It will be convenient to define the plant at the time of commencement of the attack, and then describe separately the effect of the attack upon stem, leaves and ear.



Text-fig. 1. Diagram of shoot of barley. 1. The ear-bearing internode. 2–6. The lower internodes of the stem. *A–F*. The leaves.

At the time when the egg causing the attack was laid the plant may be supposed to have been as represented diagrammatically in Text-fig. 1.

At the base there is the usual very short internode which has entirely finished its growth (6). Above this there are two internodes 5 and 4 which are well grown and have finished their growth. Internodes 3 and 2 are still undergoing active growth as are also the ear and the very short ear-bearing internode 1. Leaves *A*, *B* and *C* which belong respectively to internodes 1, 2 and 3 may also be supposed to be in active growth whereas leaves *D*, *E* and *F* belonging to internodes 4, 5 and 6 may be supposed to have finished their growth.

The effect of the larva on the plant is to cause an apparently almost immediate cessation of growth in length; internodes 4, 5 and 6 having already completed their growth are not affected in any way. The larva as it migrates downwards, eats away the young grain along one side of the ear, and then passes on to the ear-bearing internode (1) and eats a food groove down one side of this. The food groove increases in size and depth as it passes downwards, owing to the growth of the larva; it ends at the base of the ear-bearing internode. When about to pupate the larva turns round in its groove and migrates upwards a longer or shorter distance before pupating, travelling in the groove which it has already made. It is difficult to be absolutely certain whether feeding does or does not go on during this upward migration, but probably it does in some cases as a few instances of ear-bearing internodes have been seen with two food grooves—one which is narrow and shallow above and gradually becomes more extensive below and is therefore the true food groove of the downwardly migrating larva, and another shallow and broad groove branching off from some point of the former. This latter groove is made on the upward migration by a larva which leaves its old food groove. In any case the feeding on the upward migration is very slight in amount.

Only those leaves which are in active growth when the attack commences are deformed in consequence of the attack, and the leaves in the typical form of attack are not nearly so greatly deformed as those of some of the less typical forms to be described below. The reason for this is simply that in the typical form all the leaves, even (A) in the plant under consideration, have already completed most of their growth when the attack starts. The visible part of (A) is usually somewhat twisted and one-half of the lamina is often very much narrower and less developed than the other. This is, I believe, due to one side of the base of the leaf having been slightly injured by the larva. Leaf (B) shows rather less distortion and leaf (C) less still. (Pl. IV, fig. 3.)

As the larva eats its way down one side of the ear it completely destroys the rudiments of grain on that side. The effect upon the grain on the undamaged side of the ear varies considerably: sometimes the grains on this side are apparently sound, sometimes apparently sound but destitute of awns, sometimes very badly shrunken. In spite of careful measurements of large numbers of attacked shoots, I can offer no definite explanation of the above variation though I believe it is probably correlated with the state of the grain on the undamaged side when the larva commenced feeding on the ear-bearing internode, and with the amount of damage done to the ear-bearing internode and the consequent disturbance of the food supply to the ear.

Up to the time of pupation the parts of the plant upon which the larva is feeding tend to remain green and sappy though this effect is not so marked as it is for the winter generation. Very shortly after pupation the ear-bearing internode becomes hard and brittle and any grains on the ear commence to set and ripen. They cannot, of course, ripen properly except in those exceptional cases where the ear succeeds in partially escaping from the sheathing leaf.

Variations in the typical form of summer attack have to do mainly with the ear and the ear-bearing internode. The degree of stunting of the internodes below the ear-bearing internode varies, of course, considerably, according to their state of growth at the time when growth was inhibited by the larval attack; the length of the ear-bearing internode and the size of the ear also varies considerably for the same reason.

Besides being stunted the ear-bearing internode may or may not be considerably thickened; it is also frequently waxy and twisted instead of straight. Occasionally the food groove does not extend to the base of the ear-bearing internode, and in three cases out of many hundreds examined, the food groove extended below the ear-bearing internode on to the stunted internode below it. The damage done to the ear varies considerably according to the stage of growth of the ear when attacked; if it was very young not a vestige of grain may develop on either side. Occasionally an ear which was well grown when attacked and is consequently not very badly damaged, will partially escape from the sheathing leaf and a few grains will ripen.

Certain special cases of damage due to the summer generation will be considered later in connection with the action of certain manures in reducing the infestation of a crop by gout fly.

Atypical forms of attacked shoots.

(*a*) *Winter forms.* When winter barley is harvested and the land left to clover many self sown plants and late tillers of barley will spring up and some of these will form a definite ear before growth ceases in the autumn. Such eared shoots may serve as winter hosts for *Chlorops* and the damage resembles precisely the typical form of summer damage.

Attacked couch grass (Pl. III, fig. 2) usually resembles the typical winter form of attacked barley; sometimes, however, a stem of couch grass with two or three internodes is attacked. In this case the basal internodes are entirely unaffected while the apical part which is affected resembles in all respects the typical winter form, except that it arises from a long stem.

(*b*) *Summer forms.* The typical form of summer damage is produced on shoots which are fairly well grown and have a moderately well developed ear when the attack commences. If barley is late sown, or if its early growth is checked, many shoots will be attacked when practically no stem formation has occurred and when the ear is still very small. Such an attacked shoot will resemble precisely the typical winter form; the ear will be entirely destroyed and no stem formation will occur

(Pl. IV, fig. 3). There is every gradation between this form and the typical summer form. Thus an attacked shoot may be like the winter form except that it arises from one, or two, or three, short basal internodes (Pl. IV, figs. 4 and 5); it may contain a small badly damaged ear or the ear may be entirely destroyed. The form of summer attack on couch grass is the same as that of the winter form; there is no form on couch grass resembling the typical summer form on barley.

The damage on wheat, both of the winter and summer generations, resembles that on barley except that so far no summer type damage has been found on wheat in winter, and it probably does not occur as the wheat harvest is too late to allow self-sown wheat to form ears before winter.

For practical purposes a shoot of barley attacked by gout fly may be considered as totally destroyed, but it is important to discover whether the effect ends there, or whether an attack upon one shoot of a plant has any bad effect upon the yield of other non-attacked shoots of the same plant. It may be said definitely that there is no visible effect. In 1922 several small experimental plots were set up. When harvested the plants were pulled up by the roots. The length of straw of every shoot and the number of sound and unsound grains in every ear were then obtained. This body of data seems to settle quite definitely that if one shoot of a plant is attacked, the other shoots are not thereby affected in any way.

The distortion of the plant produced by *Chlorops taeniopus* is in several respects extremely interesting. Unfortunately I have not as yet found time to examine sections of the plant tissue in order to determine the precise nature of the distortion as regards the individual cells. The malformation caused by *Chlorops* is, as far as I have seen, quite unique amongst grass feeding larvae, and it is unique in this respect; that the *Chlorops* larva produces distortion of parts of the plant with which it is not actually in contact, which it does not damage and which are below the region of damage and whose food supply cannot therefore be affected. This fact can be made out in almost any "gouty" shoot—with difficulty in some of the winter type damage—but is most clearly seen in some of the non-typical forms of summer damage on barley where, as already mentioned, the degree of distortion of the leaves is considerably greater than in the typical form. In such a shoot one may find a leaf arising from a node separated from the region of actual damage by one, two or even three internodes, and yet showing distinct and often very prominent signs of deformation. It is, in fact, obvious, that any region which is actively growing when the larva commences feeding is affected, and the more active its growth at the time, the greater the distortion produced. As a general rule the younger parts are the most rapidly growing parts but there is probably one exception to this. The apical or central leaf of a shoot suffering from either the typical form of winter attack or a non-typical summer attack is very rarely as much distorted as the two leaves next below it, which are usually the most distorted leaves on the plant; it ceases to grow in length and usually becomes spirally curled but it does not become noticeably thick or broad. This is quite explainable if the growth of the leaves follows the usual growth curve which is typical for living individuals and parts of living individuals, *i.e.* rate of growth starts slowly, attains a maximum and then diminishes. The central leaf, being the youngest, will probably not have attained its maximum rate of growth when the deformative influence sets in, whereas the two leaves below it will more probably be nearly at their maximum growth-rate. In the case of the typical summer form, the attack takes place when the plant is in a more advanced stage of growth, and in this case it is the apical leaf which is nearest its maximum growth-rate—very frequently even the apical leaf has apparently passed the maximum growth-period and its growth-rate is declining when the attack takes place; hence the relatively slight distortion of the leaves in this form. I cannot at present say whether the increase in breadth and thickness of the leaves is due to increase in cell size or cell numbers. Badly deformed leaves undoubtedly give the impression of being in a state of excessive turgidity and on general grounds it seems that increase in cell size is more likely than increase in cell numbers. It has been stated above that the commencement of larval feeding inhibits growth in length. This is not strictly accurate; there must be a slight amount of irregular growth in length of the leaves to cause the twisting of their laminae and the puckering of their edges. With regard to the stem the attack has little effect beyond inhibiting growth in length of those internodes which are growing. Occasionally some of the internodes show slight twisting or bending (indicating the occasional survival of slight, irregular, growth) and may become slightly thickened.

It seems to me that the only way to explain the deformative influence of the larva upon parts of the plant below the actual damage is to suppose that the larva introduces some substance into the sap which is distributed throughout the plant and that it is this substance which causes the deformation of the growing parts of the plant. If this is so the substance in question is probably formed in the salivary glands of the larva. An attempt was made to test this point by making an extract of several larvae in salt solution and injecting this into the plant. This experiment was done before the importance of the growth-rate on the resultant malformation was understood, and I now believe that the plants selected for the purpose were too old, and that the failure to produce the malformation artificially was largely a result of this. The difficulties of injecting liquids into the plant tissues are, of course, considerable, but I believe that artificial malformation would be produced if the larval salivary secretion could be introduced into the plant sap.

If a larva is removed from a shoot in which it has been feeding for some time, or if, for some reason, it dies within the shoot, growth does not recommence. In the case of a typical form of winter attack growth is, of course, impossible, because the young rudiment of stem and ear has been entirely destroyed. In the case of a typical summer attack from which the larva has been removed, this explanation does not hold good: it seems rather that growth is entirely and permanently inhibited. Certain exceptional cases, where the larva enters a shoot too far advanced in growth to allow the larva to develop, will be discussed later in connection with the action of manures and early sowing upon the infestation by gout fly.

The advantage to the larva of the deformation produced is fairly obvious, particularly in regard to the typical form of summer attack. The inhibition of growth prevents the ear-bearing internode from lifting the ear out of its sheathing leaf and so exposing the larva to the open; if this happened the larva would almost certainly fall off the internode as it has no adaptations for maintaining a firm hold upon a dry, smooth and vertical support. So long as the internode remains within its sheathing leaf the larva is kept safely pressed between the internode and the leaf. In some way, as already mentioned, the larval feeding has the effect of keeping the tissues green and sappy and therefore in a suitable condition for the larva. In the winter type of attack this latter function of the deformative influence is probably the more important, as the question of growth does not arise, the larva having destroyed the stem rudiment. It is remarkable upon how small a shoot a larva can develop. A small shoot of couch grass, of less diameter than the full grown larva, may be attacked; as the larva develops the leaves thicken, and the shoot becomes thicker and thicker and is apparently robust and flourishing although stunted. Thus winter larvae have been found in shoots of couch and wheat less than 1 in. long, but relatively enormously swollen. It seems probable that when a larva is in a very small shoot the latter is stimulated to a degree of assimilation quite out of proportion to its size and in this way the *Chlorops* larva can develop in shoots initially smaller than could be used by a smaller larva such as *Chloropisca.* If very small shoots are attacked by *Chloropisca* or Frit they quite frequently die off before the contained larva can mature: these larvae are, however, quite active and it is possible that they can leave an unsuitable shoot and enter another. I have at all events succeeded in removing them from one shoot and transferring them on to the apical leaf of another close to the ligule, and in several cases they have entered the new shoot and completed their development there. I have never succeeded in doing this with *Chlorops* and I feel certain that it never occurs in natural conditions. Thus if the young *Chlorops* larva entered a very small shoot and killed it, the larva itself would die, and its method of using such small shoots by causing hypertrophy has opened up for the larvae a new source of host plant.

THE LIFE CYCLE IN ITS RELATION TO DIFFERENT HOST PLANTS.

	1922	1923
First puparium of winter generation found	March 2nd. 75 % had pupated on March 25th	March 8th. 25 % had pupated on April 4th
First fly emerged from winter generation	May 22nd	May 26th
Last „ „ „	June 10th	June 25th
First puparium of summer generation found	June 30th. 77 % had pupated on July 22nd	July 12th. 26 % had pupated on July 18th
First fly emerged from summer generation	July 31st	August 10th
Last „ „ „	September 29th	?

The flies emerging from the 1921–22 winter generation laid almost exclusively upon barley. For some time it was thought that couch grass did not serve as a summer host but a few damaged shoots of couch grass were found in that year, and a considerable number were found this year (1923).

Gouty wheat was only found in one field (New Zealand Field, Rothamsted) in the summer of 1922 and has not been found at all in 1923. The reason that the wheat in this field was attacked was that it made extremely poor growth during the winter and early spring and was extremely backward at the time when oviposition was taking place. In the majority of cases winter wheat is too far advanced in growth for oviposition upon it by the flies emerging in spring.

The flies emerging from the summer generation can utilise six types of host, and the type utilised depends mainly on the time at which the flies emerge. In 1922 the flies were emerging from the beginning of

August to the end of September, though the great majority emerged between Aug. 8th and Aug. 24th. The following are the different types of host plant used.

(*a*) Couch grass, which is always available and forms the chief winter host, and which is probably the only host available for the earliest emerging flies. An interesting point about the infestation of couch grass is that couch grass on an arable field, more particularly that growing on a field of roots such as swedes or turnips, appears to be far more heavily attacked than does couch grass growing among other grasses in hedge rows, etc. Possibly the attraction of the couch grass for the fly, perhaps aromatic, is swamped and lost when the couch grass is growing among other grasses.

(*b*) Late non-earbearing tillers of spring barley coming up after harvest. Although such tillers exist plentifully before harvest the fly does not appear to seek them out among the bases of standing corn and even if it did so many of these tillers would be cut down at harvest time and the larvae consequently destroyed. This type of host is therefore only of use for late emerging flies and when the harvest is late, as it was in 1922, the infestation is negligible and may be considered as non-existent.

(*c*) Self-sown barley due to grain shaken from the ears of spring barley at harvesting. This is an even later growth than the last class and can probably be entirely disregarded unless the harvest is quite exceptionally early. A small amount of infection of this type of host was found in the winter 1921–22 but none was found in the succeeding winter.

(*d*) Late tillers growing up after the harvesting of winter barley, and self-sown barley due to grain falling from the ears of winter barley during harvesting. The harvesting of winter barley being very much earlier than that of spring barley this is a much earlier type of growth than (*b*), and is a real danger, ranking next after couch grass in the number of larvae which it carries through the winter. I imagine that it is actually preferred to couch grass as a winter host but owing to the relatively small amount of winter barley grown (in this district) it is fortunately not very abundant.

Hosts in classes (*b*), (*c*) and (*d*) are only of danger when the barley is followed by a clover ley so that the barley shoots are allowed to stand all winter. If the land is ploughed in, or if the clover is cut for fodder during the winter, the larvae within the host shoots are destroyed.

(*e*) Autumn sown barley and wheat may be attacked if they are sown sufficiently early. In 1922 flies in captivity could not be induced to lay after the middle of October and it is probably safe to say that oviposition does not go on after that time in the open; and in any case only a relatively small number of late emerging flies will be about at this time. Therefore if winter wheat or barley are to be infected they must be sown sufficiently early to be moderately well grown plants by the middle of October. This would mean sowing in September which is a very rare occurrence. Consequently infection of this type of host is (in this district) negligible.

(*f*) There are usually numerous self-sown barley or wheat plants round a straw stack, or on a place from which a straw stack has been removed, and such shoots are frequently suitable for the winter generation. In one instance a wheat stack had apparently been removed early in the autumn. Numerous grains of wheat germinated and provided hosts for the oviposition of some of the late emerging flies from the summer generation. The shoots were extremely small when attacked but the larvae within succeeded in attaining maturity. There was no winter wheat or barley which had been sown sufficiently early for the oviposition of these flies and all the neighbouring arable fields had been ploughed in after the cereal crop harvests so that couch grass would ordinarily have been the host for these flies. The young wheat plants were, however, concentrated in one spot and their attraction to the fly was probably therefore greater than that of scattered couch grass; consequently they were heavily infected.

THE POSSIBILITY OF AN INTERMEDIATE GENERATION.

In 1922 the first fly bred out emerged on July 31st. Some of the very early emerging flies were made to oviposit on shoots of barley and couch grass to discover whether an intermediate generation of flies could be reared. The resulting larvae, however, passed the winter in the larval state and pupated next spring at about the same time as larvae derived from eggs laid some weeks later. It seems improbable therefore that an intermediate (autumn) generation normally occurs in this country, though one might possibly be produced artificially by keeping the infected plants in conditions of temperature and light approximating to the summer conditions.

HABITS OF IMAGINES; OVIPOSITION EXPERIMENTS.

The numbers of ♂s and ♀s are approximately equal. Thus of 1461 flies bred from the summer generation in 1922, 701 were ♂s and 760 were ♀s. Copulation may take place within a few hours of emergence and before the wings of the ♀ have expanded; it has never been seen to occur before the wings of the ♂ had expanded. Oviposition sometimes commences four, but more usually five days after copulation. The flies feed readily upon sugar solution or upon umbelliferous flowers but their natural feeding habits are not known; they are almost certainly flower feeders. The flies are fairly strong on the wing but in dull weather they are extremely sluggish, not readily taking to flight, and sometimes remaining stationary for hours. In bright sunny weather they become extremely active, and it is the brighter light rather than the increased heat which causes the difference. The ♀ will lay very few eggs in dull weather.

A number of experiments were carried out upon the oviposition of the flies emerging from the 1922 summer generation. A number of facts were brought out and it was hoped to amplify and corroborate these by means of experiments on the flies emerging in the spring of 1923. These flies were rather late in emerging but the experiments might still have been carried out but for the dull weather at the time, during which the flies would not lay. The barley had been planted in pots to be ready at a definite time but the delay of the flies in ovipositing allowed the barley to reach an advanced, and therefore unfavourable stage of growth before the advent of bright weather which stimulated the oviposition of the flies. Couch grass could have been planted and the work carried out on this, but by this time it was essential to turn attention to other parts of the investigation and consequently the 1923 oviposition experiments had to be abandoned. The conclusions are therefore based entirely upon the experiments done with the flies emerging in the autumn of 1922, and a few experiments done with the flies emerging in the spring of 1922.

For most of the experiments a ♂ and a ♀ were captured on the day of emergence and tubed together, with sugar solution for food; for some experiments no food was given, but only water. After fertilisation the ♀ was removed to the oviposition chamber, the ♂ being left in the feeding tube (which was not corked but covered over with cotton gauze). The number of eggs laid was counted, when possible, every day, and the ♀ removed to fresh shoots when necessary. Some flies had filter paper moistened with sugar solution in their oviposition chambers, others had filter paper moistened with water only. For experiments with unfertilised ♀s, puparia were tubed separately and the flies bred out so that there was no possibility of pairing. In some cases the ♀s were confined in a gauze cover covering over a whole pot in which couch grass or barley was growing but in other cases the flies were confined in a lamp chimney closed with gauze at the top, and covering one or two shoots of couch grass or barley. The following are the chief conclusions drawn from the experiments:

(1) Pairing may take place on the same day as emergence and oviposition commences sometimes four, but usually five days after pairing.

(2) The greatest number of eggs laid by a single ♀ in captivity was 147, but several ♀s laid over 100.

(3) Unfertilised ♀s commence laying 10–12 days after emergence. Fed on sugar solution they lay about 12 eggs; no unfertilised ♀ fed on water alone laid more than three eggs[1].

(4) Unfed fertilised flies lay about half as many eggs as fertilised flies fed on sugar solution.

(5) A fed and fertilised ♀ may lay up to 17 eggs in 24 hours. An unfed fertilised ♀ only lays up to 7 eggs in 24 hours.

(6) More than one act of copulation is necessary to fertilise all the eggs that a ♀ is capable of laying. A ♂ which has fertilised a ♀ once is capable of again fertilising the same or another ♀.

Eggs were laid in greater numbers and with greater rapidity by the flies confined beneath glass lamp chimneys than by those confined under gauze covers. In the former case the flies are subject to higher temperature, greater humidity, and greater light intensity than in the latter. One of the 1923 experiments which had to be abandoned was designed to disentangle roughly the relative values of these three factors.

It is a striking and important fact concerning the flies that the ovipositing instinct of the females is not perfect. They usually select shoots of a certain type of growth, *i.e.* moderately well grown but with little development of the ear. Here their instinct is seldom at fault and they do not often lay upon a shoot which is inherently unsuitable. As a general rule eggs are laid in the field upon one of the four uppermost leaves but sometimes they are laid on the very lowest leaves where there is no chance for the young larva to develop. As will be shown later there is frequently a very considerable difference between two adjacent leaves (*e.g.* the second and third from the apex) as regards the chances of success of larvae from eggs laid upon them. In such cases the fly lays almost as frequently upon the less favourable leaf as upon the more favourable one.

The length of life of the flies has been determined only for flies emerging from the summer generation. In 1922 some flies which emerged on August 5th were placed in a large glass cage with a wire gauze top and the cage was placed in an insectary facing north so that it would not be subject to excessive sun. Pieces of filter paper moistened with sugar solution were given as food. On October 10th the flies were still alive and were removed and given the opportunity of ovipositing on couch grass. (The flies had laid a considerable number of eggs on the glass sides of their cage, where they had not been provided with any host plant.) Oviposition continued up to October 13th and the eggs laid were fertile and gave rise to normal winter larvae.

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[1] Such eggs do not, of course, hatch.

On October 16th it was noticed that the flies were becoming lethargic and were crawling about the soil in the lamp chimneys in which they were placed for oviposition, instead of seeking the top. They had laid no eggs for three days. These flies were then transferred to a dark chamber containing sphagnum moss and food in the form of filter paper moistened with sugar solution, and the dark chamber was left in the insectary over winter to discover whether the flies would hibernate. When examined on June 4th, 1923, all the flies had died. This is not, of course, a proof that hibernation does not occur, but hibernation is improbable.

The length of life of flies emerging in spring probably does not usually exceed a fortnight.

EFFECT OF MANURIAL TREATMENT UPON THE INFESTATION OF BARLEY BY GOUT FLY.

It has frequently been recorded that well manured barley fields suffer less from gout fly attack than do poorly manured fields. Hoos Field on Rothamsted Experimental Farm gives an opportunity of examining this question critically because here there are permanent barley plots which have received the same manures for many years. All these barley plots are strictly comparable except for the different manurings; they are side by side on the same type of soil and are sown at the same time.

The method used for comparing the infestation of the different plots was as follows. On each plot 10 counts were made, each of 100 shoots, and the number of infested shoots in each count of 100 was noted down. This gives for each plot 10 percentage figures of infestation, and the mean of these ten values is taken as the figure of infestation for the plot. The actual damage done to the crop is in all cases slightly higher than the figure given because the counts included all shoots, and not only those which would form ears at harvest time. The counts were done in this way because they were done some time before harvest in 1922 and the distinction between a shoot which is going to produce an ear, and a shoot which is not going to do so, is not always clear cut and definite. To attempt this distinction would therefore have introduced an error due to personal judgment and this error might not have been constant for the different plots. Before starting on the counts on the plots it was necessary to test whether in a uniform field of barley the infestation was uniform over the whole field or whether it was greatest at the edges and diminished towards the centre, the latter of which might perhaps at first sight be expected since the flies come into the field from outside and are not originally scattered all over the field. Tests on two barley fields on Rothamsted Experimental Farm (Long Hoos Field and Stackyard Field) showed, however, that the infestation was uniform, if the headlands were excluded. On the headlands the infestation was slightly higher than within the field owing to poorer cultural conditions of the headlands in general, and some parts of them in particular. The uniformity of the infestation is due to the facts that the ♀ fly is active, and may continue oviposition for over a fortnight, and the fly definitely selects shoots of a certain type of growth for its oviposition and these favourable shoots will be scattered all over the field.

In presenting results obtained from Hoos Field it will be convenient to give first the general conclusions arrived at in 1922. I do not propose to give the full data of these manurial counts as this will be found in the statistical survey of the figures by Mathetes(5). It was found that manuring had a very marked effect upon the infestation; well manured plots being markedly less infected than unmanured plots. Farmyard manure was very effective in reducing infestation; of the artificials, superphosphate and potash were the two manures having the greatest effect in reducing infestation; $(NH_4)_2SO_4$ had some effect when acting in conjunction with some manures but no effect when acting alone, or with certain other manures. A more detailed analysis is given later. The two extremes of the series in 1922 were plot 6_1 (unmanured) with an infestation of 19·1 per cent. and plot 5A (K_2SO_4 + Super. + $(NH_4)_2SO_4$) with an infestation of 4·8 per cent.

In 1922 therefore it was definitely established that manuring had an effect upon the intensity of attack of gout fly, but exactly how this effect was produced was still unknown and it was necessary to consider all the possible ways in which the manures might be acting with a view to testing these in 1923. The possibilities may immediately be divided into two classes. Considering the two plots 6_1 (19·1 per cent. infected) and 4C (complete minerals and rape cake, 5·1 per cent. infection) the possibilities are:

A. The flies lay fewer eggs per unit number of shoots on plot 4C than on plot 6_1.

B. The flies lay the same number of eggs per unit number of shoots on both plots, but a large percentage of them fail to develop on plot 4C.

To consider A first:

If fewer eggs are laid on plot 4C than on plot 6_1, there are two possible explanations:

(1) The manurial treatment forces on the plants so that by the time egg laying commences (*i.e.* early part of June) they are in an advanced stage of growth unfavourable for oviposition.

(2) The manurial treatment may in some way alter the constitution of the plant so that its attraction for the fly (possibly aromatic) becomes less. Although this has to be considered as a possibility it is undoubtedly an improbable explanation.

If B is found to be correct there are again two possibilities:

(1) The plants on 4C may develop so rapidly in growth that by the time the eggs hatch the plants may be unsuitable (*e.g.* too tough) for the young larva, or the ear may force itself out of the sheathing leaves before the young larva can attack it.

(2) It is possible that something in the constitution of the cell sap is actively injurious to the larva and causes the death of many of them in the early stages before they have had time to inhibit the growth of the plant.

It may be said at once that B (1) is essentially correct and explains most of the observed differences. A (1) is also correct and is responsible for some part of the differences. A (2) has not been tested directly but is almost certainly incorrect. An experiment comprising 70 pots containing barley under different manurial treatments was set up in the spring of 1923 in order to test B (2). All pots were confined under a large gauze cover and gout flies were introduced into the cage. Unfortunately, as already explained, the flies would not lay during the dull weather and the plants continued to grow and became unfavourable for oviposition. The experiment consequently failed and need not be described in detail. It was hoped that by taking one infected shoot every day from each type of manure and dissecting it, that the influence (if any) of the manurial treatment upon the larva itself as apart from the influence upon the plant, could be followed out. In spite of the failure of the experiment I am convinced from a careful examination of infected shoots from certain of the Hoos plots (see later) that the manures have no injurious action upon the larva except in their effect on the plant growth.

For pointing out the possibility of the following two criticisms of the 1922 infestation counts on Hoos Field, I am indebted to Dr A. D. Imms and Mr R. A. Fishèr respectively.

(1) If any manure causes, after oviposition is finished, the development of numerous late tillers, the percentage of infestation will be reduced as these will be included in the counts.

This point was tested in 1923 by counting on seven of the plots (see Table I) the number of shoots per square yard at the time of oviposition and the number per square yard at the time when the infestation counts were made. Also the number of shoots in marked strips upon certain of the plots were counted at the same time. The former is probably the more accurate test as it represents the condition of the plot in general and not of just one part of it. The results show that the infestation counts are not invalidated by the late growth of tillers. On two of the plots (4C and 5A) the surprising result was obtained by both the above methods that there were fewer shoots at the time when the infestation counts were done than there were at the time of oviposition. This must be due to the dying down of very young tillers on these plots, the small dead and shrivelled tillers being missed in the second series of counts of shoot numbers. Some were perhaps killed by insect attack (*e.g.* frit fly or *Chloropisca circumdata*). I do not think that the death of these young tillers on 4C and 5A will affect the validity of the infestation counts (or rather the conclusions to be drawn from these—they certainly do not affect the actual counts), but it is a difficult point. Some of the young tillers which died doubtless bore eggs, but the percentage of them bearing eggs was probably the same as the percentage of the total shoots of the plot bearing eggs. In this case the death of the young tillers would affect the accuracy of neither the egg counts nor the infestation counts.

(2) Certain manures appear to have the effect of reducing infestation; but these same manures may also have the effect of increasing the number of shoots per unit area. Suppose that the fly is evenly distributed and lays absolutely at random, and that the infected shoots are consequently evenly distributed per unit area. On the plots with the most shoots per unit area the percentage infestation of the shoots will be lower than on the plots with the least shoots per unit area.

This point was tested in two ways. Firstly, the egg counts showed that the number of eggs per unit area was not the same for all plots. Secondly, the number of shoots per square yard was counted on all the Hoos Field plots when the infestation counts were made. These figures show quite definitely that the differences in the infestation of the various plots cannot be accounted for by the differences in the numbers of shoots per unit area on the plots, EVIDENCE RELATIVE TO THE EFFECT OF MANURES ON OVIPOSITION.

On June 18th, 1923, samples of the plants were taken from plots 6_1, 7_2, 1A, 5A and 4C. For a sample of any one plot a few plants were pulled up at twenty different points on the plot. The samples were brought into the laboratory and each was examined separately for eggs. In this way both sides of every leaf in a sample were examined which would be impossible in the field. On July 2nd counts were made on these five plots and one other (plot 1AA) of the number of shoots per square yard. A yard stick was thrown on to the plot and then laid alongside that row of barley to which one of its ends (which was marked) lay nearest, and the number of shoots along a yard length were counted. This was done in five different parts of the plot and as the rows of barley are 7 ins. apart the total of the five counts gives the number of shoots per square yard. From these six counted plots samples were taken large enough to contain more shoots than would be found in a square yard of the plot in question. These were brought into the laboratory and examined for eggs as previously, except that only sufficient of each sample was examined to give the number of shoots per square yard of the plot from which the sample was taken. Thus the actual number of eggs per square yard was obtained. In the case of the first count the number of eggs per square yard was

Table I.

First Egg Count. June 18th to 26th (samples taken on June 18th).

	Plot	Infestation %	No. of shoots per sq. yd. at time of egg count, July 2nd	No. of shoots per sq. yd at harvest	Percentage of shoots bearing eggs	No. of shoots per sq. yd. bearing eggs	No. of eggs per sq. yd.
Unmanured	6_1	14·1	225	239	20·6	47	55
Dung	7_2	4·3	463	382	15·1	70	74
$(NH_4)_2SO_4$	1A	14·2	277	258	18·9	52	59
Super. $+ K_2SO_4 + (NH_4)_2SO_4$	5A	9·1	372	243	17	63	69
Complete minerals + Rape Cake	4C	5·9	366	315	9·5	35	42
Second Egg Count. July 2nd to 10th (samples taken on July 2nd).							
Unmanured	6_1	14·1	225	239	31·5	71	88
Dung	7_2	4·3	463	382	9·5	43	43
$(NH_4)_2SO_4$	1A	14·2	277	258	18·8	52	60
$NaNO_3$	1AA	16·5	274	299	17·5	48	55
Super. $+ K_2SO_4 + (NH_4)_2SO_4$	5A	9·1	372	243	14	52	56
Complete minerals + Rape Cake	4C	5·9	366	315	6	22	23

calculated from the number of shoots examined and the number of shoots per square yard as obtained on July 2nd; some samples included more and others less shoots than would be found in a square yard of the plot from which they were taken. Table I sets out the results of these two examinations.

The plots selected were those which showed some of the highest and some of the lowest infestations in 1922. A very unfortunate omission was made in that plot 2. O. (manured with superphosphate only) was not examined, as superphosphate is the most effective of the artificial manures in reducing infestation. All samples, to be comparable, must be taken on the same day or the samples taken later will be exposed to the chance of oviposition longer than will the earlier ones. I am indebted to Dr Imms for the advice, when I was about to start the second count, to repeat it on the plots which I had already done instead of doing an entirely fresh series; several interesting points would have been missed had I done this. All samples were taken on the same day and stood in water until the examination was finished. If the samples are done one at a time it is possible that some of the eggs will fall off the plants, and if so obviously a greater percentage will have fallen off on the samples examined last. I do not believe that the eggs do fall off under these conditions but to avoid the possibility of this error a portion of each sample was done each day until the whole count was finished; this occupied slightly over a week, and by the end of this period the plants were commencing to become mouldy. It was owing to these difficulties that only six of the Hoos Field plots could be examined.

The manuring of the plots undoubtedly has some effect upon the degree of oviposition upon the plants, and the degree of oviposition shows a distinct correlation with the final figures of infestation as is shown if the plots are arranged in order of descending magnitude as regards the percentage of shoots bearing eggs and the final infestation figures for 1923. It is equally obvious that the difference in the degree of oviposition on two plots does not explain all the difference between the final infestation of the two plots, and that on some plots a greater percentage of eggs failed to produce infection of a shoot than on other plots.

Comparing the two counts, the percentage of shoots bearing eggs is practically the same in both for plots 1A and 5A. The percentage on 7_2 is materially bigger in the first than in the second count. The plants on 7_2 grow rapidly, and at the time when the first count was made they had probably passed the stage of growth favourable for oviposition. After this few or no eggs would be laid on this plot, and a certain number of eggs had hatched and fallen off by the time the second count was made, thus lowering the numbers. The same thing is seen in plot 4C, another rapidly growing plot. Plot 6_1 shows a much bigger percentage of shoots bearing eggs in the second count than in the first. Growth is slow on this plot and it is probable that between the first and second counts more and more shoots reached a stage favourable for oviposition. Thus plots 7_2 and 4C are normally only used by early emerging flies, whereas plots such as 6_1 are also available for later emerging flies. A certain number of eggs would, of course, fall off on all the plots between the two egg counts, but the relative numbers falling off would almost certainly be the same for all plots. On plots 1A and 5A those falling off were just about balanced by fresh ovipositions.

By comparing the final infestation of a plot with the percentage of shoots bearing eggs (in all cases the greater percentage figure of the two counts is taken here as representing more nearly the maximum oviposition upon a plot) one can obtain a figure representing the percentage of the number of shoots bearing eggs which eventually become infected. This figure is admittedly only approximately accurate. The figures are: 1AA, 94·3 per cent.; 1A, 75·1 per cent.; 6_1, 42·2 per cent.; 5A, 53·5 per cent.; 4C, 62·1 per cent.; 7_2, 25·5 per cent.; the series being arranged in order of descending magnitude of their final infestations. Thus on plot 1AA practically every shoot bearing an egg becomes infected, while on plot 7_2 only about one in every four shoots bearing eggs becomes infected. The two plots do not differ very greatly in their early growth and in their consequent liability to oviposition. They do differ greatly in their relative numbers of critical leaves (see below), and in their later growth, and consequently in the chance of a larva from a given egg infecting a shoot and attaining maturity.

Plot 6_1 is probably the slowest growing plot on Hoos Field and one would therefore expect a greater percentage of eggs to develop than is shown in Table I[1]. I regard with considerable distrust the explanation of the discrepancy which I give below as it is almost entirely conjectural and is not rigidly based upon either observation or experiment. The ear-bearing shoots on plot 6_1 are in many cases extremely small and the size of a shoot is no index to its degree of maturity. The oviposition instinct of the fly is undoubtedly imperfect since it frequently lays eggs where, from the very beginning, and apart from the later growth of the shoot, the larvae emerging from the eggs have no chance of developing. It is possible that on plot 6_1 the fly very frequently lays upon such unsuitable plants. It must be pointed out that what occurs on plot 7_2 is in the main entirely different. Here the shoots selected for oviposition are usually suitable at the time when oviposition occurs, but during the incubation period of the egg the shoot grows so rapidly that by the time the larva emerges from the egg the plant is unsuitable[1].

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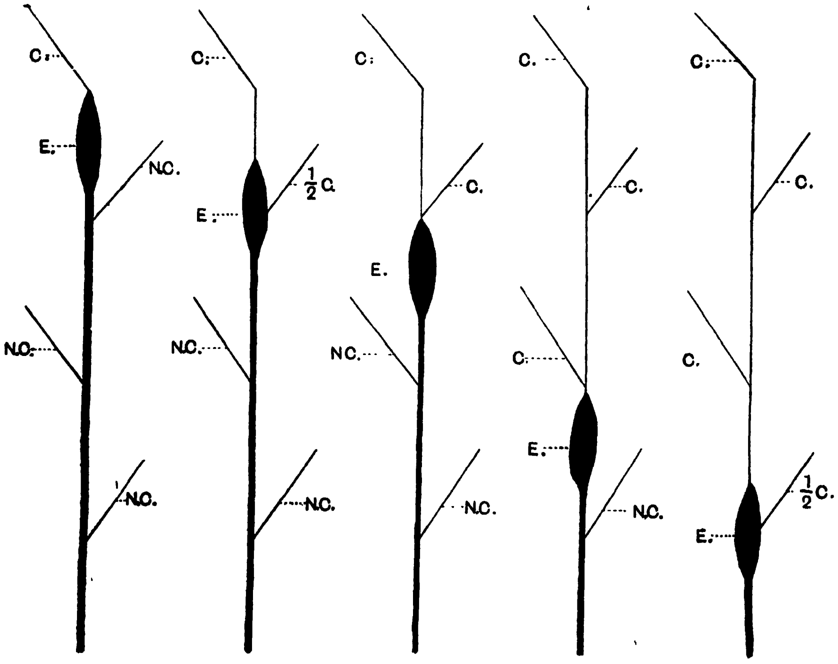
[1] As is shown later (p. 205) very few larvae are killed by actual rapidity of plant growth on this plot.

EVIDENCE RELATIVE TO THE EFFECT OF MANURES UPON THE LARVAE.

The following theory as to the action of the manures upon the larvae was formulated at the time when the egg counts were done in 1923. It will best be understood by reference to the diagrammatic Text-fig. 2. The whole question turns upon the position of the ear with regard to the leaves; if an egg is laid upon a leaf lamina whose base comes off from the shoot below the base of the ear, the first instar larva will enter the shoot below the ear. If the egg is laid upon a leaf arising very low down upon a shoot



Text-fig. 2. Diagram of barley shoots showing, from left to right: 1, 1½, 2, 3 and 3½ critical leaves. *C* = critical leaf; ½*C* = half critical leaf; *E* = ear; *N.C.* = non-critical leaf.

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[1] 7_2 also differs from 6_1 as regards critical leaves (see below).

the young larva will even reach the plant below the ear-bearing internode. It is an invariable rule that when a larva enters the shoot it moves downwards, and therefore if it enters below the ear it can never get on to the ear to feed. All leaves above the ear of a shoot may therefore be termed the "critical leaves," *i.e.* a larva from an egg laid upon any of these leaves will have a chance of infecting the shoot. A leaf arising from the shoot half way up the level of an ear is considered as half a critical leaf because the larva from the egg on such a leaf would enter the plant upon the ear though not upon its tip. The ovipositing instinct of the fly is not perfect and eggs are frequently laid upon leaves arising from the shoot below the ear. It was thought that if a larva failed to get on to the ear it would die; this is not strictly accurate as the 1923 examinations have shown that a larva can feed for a certain length of time upon the ear-bearing internode alone, and in certain very rare cases it will even attain maturity under these conditions: usually it dies in the second instar or early in the third instar. These cases will be considered later but they are exceptional and do not affect the theory. Comparing plots 7_2 and 6_1:

It was supposed that owing to the greater growth on plot 7_2 there would be on the average, fewer critical leaves per shoot upon this plot than upon 6_1. Consequently a greater proportion of the eggs upon 7_2 would be laid upon unsuitably placed leaves than would be the case on plot 6_1. Further, suppose an egg to be laid upon a leaf arising from the shoot just below the tip of the ear (*i.e.* a "½-critical leaf") its chance of infecting the shoot would be greater in plot 6_1 than in plot 7_2 because growth is more rapid in the latter. The incubation period of the egg is about 8 days. During this time the ear in the shoot on plot 7_2 has a very good chance of growing up above the leaf upon which the egg is laid; the ear of the shoot upon plot 6_1 has a very much less chance of doing so.

There is one other way in which the manures occasionally react upon the larva and although very rare (according to the 1923 observations) it deserves some mention. An egg may be laid upon a leaf which is entirely suitable as regards position and the young larva may enter the shoot on, or above, the apex of the ear and yet may fail to develop because the ear, though still within its sheathing leaf, is too mature and tough for the young larva. Practically no damage is done to the plant in these cases.

Three bodies of evidence have been collected in support of the "critical leaf" theory, one experimental, which I shall therefore present first, and two observational.

The whole "critical leaf" theory could have been rigidly tested under controlled experimental conditions if the oviposition experiments had been successful. It was suggested to me by Dr A. D. Imms that it would be well worth while to try to test the point in the open although strict control would in this case be impossible. Accordingly on July 9th, 12 shoots bearing eggs were selected along one side of plot 6_1: they were selected as having eggs upon only one of the leaves. Each shoot was marked and a note made of the number of critical leaves upon each shoot and the number of the leaf (counting from the apex downwards) upon which the eggs were laid. A prediction was then made as to whether the shoot would become infected or not. At the time it was not known that a larva which failed to get on to the ear of a shoot could still damage the internode; if this had been known the predictions would have been somewhat modified.

Table II.

Shoot No.	Eggs (July 9th)	Prediction	Final observation (Aug. 22nd)
1	3½ critical leaves. Egg on leaf no. 4	May or may not be infected	Not infected
2	2½ critical leaves. Egg on leaf no. 4	Will not be infected	„
3	2½ critical leaves. Egg on leaf no. 4	„	Infected but only the internode damaged. Ear untouched
4	2½ critical leaves. Egg on leaf no. 4	„	Infected but only the lower half of the internode damaged
5	4 critical leaves (no sign of ear). Egg on leaf no. 3	Will be infected	Infected. Damage from apex of ear downwards. Shoot remained very small, not much distorted but ear almost completely destroyed
6	2½ critical leaves. 2 eggs on leaf no. 4 (one hatched)	Will not be infected	Infected. Very shallow groove down middle of internode. Internode not in the least distorted and over 9 ins. long. Larva certainly did not mature
7	4 critical leaves. Egg on leaf no. 3 (no sign of ear, apical leaf only just appearing)	Will be infected	Infected. Shoot remained small. Distortion slight. Ear almost completely destroyed
8	4 critical leaves. Egg on leaf no. 3 (no sign of ear)	„	Infected. Slight damage on internode only
9	3½ critical leaves. Egg on leaf no. 3	„	Ear had failed to come out of sheathing leaves but no certain sign of *Chlorops* damage could be detected
10	3½ critical leaves. 2 eggs on leaf no. 3	„	Definitely not infected
11	3½ critical leaves. Egg on leaf no. 4	May or may not be infected	Not infected
12	3½ critical leaves. Egg on leaf no. 3	Will be infected	Infected. Distortion not great but ear almost completely destroyed

Nos. 1, 2, 5, 7, 11 and 12 follow the prediction. Nos. 3, 4 and 6 also follow the prediction in the sense that when this was made the type of infection where only the internode is damaged was not known, and the prediction does not therefore take into account this type of damage. No. 8 follows the prediction but I cannot understand why, in this case, the damage is to the internode only. Either there was quite abnormally rapid growth in this shoot after the egg was laid or else the egg observed did not hatch and an egg laid at a later date is responsible for the damage. This is the great objection to carrying out this experiment in the open—one cannot be sure that eggs are not laid upon the shoots after the observations are made. One can be fairly sure that infection has not taken place prior to the observations because in the young shoots the very first sign of distortion can be detected by a careful examination. No. 9 is a doubtful case. No. 10 should have been infected and was not; this is the only instance where the result is directly opposed to the prediction. This was the biggest shoot and had the biggest ear of the whole twelve and it may be that this is one of the cases previously referred to where the ear, though in the right position for infection, is too mature and tough for the larva. Unfortunately birds had been feeding on this ear so that it could not be carefully examined for minute signs of attack at the apex of the ear. I think therefore that this experiment offers strong support to the critical leaf theory; it does not, and could not be expected to afford definite proof since the experiment could not be carried on under critical conditions.

Assuming the critical leaf theory to be correct, if one could at the time of oviposition arrange the plots on Hoos Field in a series according to the relative numbers of critical leaves per shoot on the different plots, the order of the series should correspond with the order of the final infestations. This test cannot be carried out really accurately because to be of any value all plots considered must be examined, as regards their relative number of critical leaves, upon the same day, and an examination of several thousand shoots on each plot would be necessary for real accuracy. An eye estimate of a single observer is valueless being liable to very grave errors, but the error is considerably less if four observers estimate the critical leaf value of a plot and the mean of the four observations is taken, and it was suggested by Dr Imms that observations might be made in this form on a few plots which were expected to give considerable differences in the final infestation figures. I am greatly indebted to Dr Imms, Mr Garner and Mr Eden for having enabled me to make the observations in this form. All four observers examined the same plot together; when the examination was finished figures were agreed upon which represented the mean of the four opinions. The following two examples may be given:

Plot 4C.	0 to 1	critical	leaves on	90 %	of the	shoots
	1½ or more	,,	,,	10	,,	,,
Plot 1AA.	0 to 1½	,,	,,	0	,,	,,
	2	,,	,,	15	,,	,,
	2½ or more	,,	,,	85	,,	,,

Plot 1AA is, from the above figures, obviously more liable to infection than plot 4C. The estimates of the four observers were usually remarkably close. Plots 1AA, 6_1, 1A, 5A, 7_2 and 4C were examined in this way and placed in the order given as regards the number of critical leaves per shoot, 1AA having the most and 4C the least. The correspondence of this order with the order of the same plots as regards their final infestation figures is truly remarkable when it is remembered that their order as regards relative numbers of critical leaves is based upon estimate only, which cannot be expected to detect small differences with accuracy.

The last body of evidence relative to the effect of manures upon the larvae and supporting the critical leaf theory is drawn from a very careful examination of infected shoots on plots 1AA, 1A, 6_1, 5A, 4C, 2. O., and 7_2. When the infestation counts were done on these plots at the end of July 1923, the infected shoots from each sub-count of 100 shoots (see above, p. 193) were brought into the laboratory and carefully examined, there being thus ten samples from each plot. It is impossible to give this evidence in full.

In many instances (36 altogether) a young larva was found dead upon the ear-bearing internode and in all these cases the internode only had been damaged. It was absolutely certain that in these cases the first instar larva had entered the shoot below the ear and had therefore commenced its feeding upon the internode where it had not been able to obtain sufficient nourishment, the internode being too tough, and it had consequently died. The damage done to the internode was usually very slight, the food groove being in some cases less than ½ in. in length. Even in these cases the ear failed to emerge fully from the sheathing leaf and the distortion of the plant, though slight, was sufficient to mark it out in the field as having been attacked. The degree of distortion of the plant varies to some extent according to the amount of damage done to the internode and this depends mainly upon the degree of hardness (*i.e.* maturity) of the internode when the young larva commences feeding. If the internode is fairly young and soft the larva may contrive to feed for some time and may even get as far as the beginning of the third instar before dying from insufficient food. Two cases have been seen in which a larva attained maturity and pupated although only the internode was attacked. Besides the 36 cases in which the dead larvae were actually found there were 69 cases of slight damage to the ear-bearing internode where the larvae were not found but were, with I think perfect safety, assumed to be dead. In examining large numbers of infested shoots (about 700 were examined on the seven plots mentioned above) it is impossible to spend time searching for a minute dead first or second instar larva if it does not happen to have died in the food groove; the dead larvae are moreover liable to fall out in opening the shoot for examination.

All the larvae causing the above type of damage must have failed to produce the typical damage to the ear for one of two reasons. Either the eggs from which the larvae emerged were laid upon a leaf arising from the shoot below the base of the ear, or else, although originally laid upon a suitable leaf, the rapid growth of the ear during the period of incubation of the egg had carried it above the leaf in question by the time the larva emerged. If this is so more larvae should be killed in this way upon rapidly growing plots than upon others and this is undoubtedly the case as the following figures show. The plots are arranged in order of descending magnitude of infestation:

	1AA	1A	6_1	5A	2. O.	4C	7_2
Larvae killed by rapidity of plant growth	15·25 %	4·2 %	9·2 %	18·7 %	35·8 %	23·7 %	25·6 %

I cannot explain the high figure on plot 1AA which is manured with $(NH_4)_2SO_4$. Excessive quantities of nitrogenous manures retard the maturity and growth of the ear and ear-bearing internode (on Rothamsted soils) and one would therefore expect few larvae to be killed by the plant growth on this plot. The expected effect of nitrogenous manures is shown in plot 1A which is manured with $NaNO_3$. The figure for plot 2. O. (manured with superphosphate) is extremely interesting, being the highest of all. It has already been mentioned that superphosphate is the most striking of the artificial manures in reducing gout fly infestation. One of the most firmly established facts regarding the effect of superphosphate upon the plant is that it hastens maturity. This is precisely what is indicated by the large percentage of larvae which are killed by the growth of plants manured with superphosphate. The number of larvae killed on the other four plots fits in very well with the relative rates of growth on these plots as far as this is known.

A particular type of damage must be mentioned here. One occasionally comes across shoots bearing an ear upon a long uninjured internode which is, however, usually twisted and bent (sometimes extraordinarily so) and arises from a basal region which looks exactly like a typical winter form of attack except for the fact that the stem and ear arise from it. For a long time I took these to be shoots which had been attacked and had subsequently "recovered," and I thought that they formed an exception to the rule that once growth has been stopped by larval attack it will not recommence. I think now that this type of deformation is due to an egg being laid upon one of the lowest leaves of a very young shoot, *i.e.* upon a leaf far too low down to be a critical leaf. In a few cases the larva might get into the base of the shoot and feed for a short time before dying on account of its unsuitable position. It may be supposed to feed long enough to inhibit the growth of the young leaves and the lower internodes of the stem which are just forming. For some reason which I cannot explain, its inhibiting influence is not sufficiently powerful to stop growth of the ear and ear-bearing internode although its influence is shown in the deformed wavy growth of the internode. Whether this view is correct I cannot say as I have never been able to find the dead larva in this type of shoot (which is very rare), and the above is not brought forward as evidence in support of the critical leaf theory. It is merely an abnormality which seems to be explained by the theory. Taking into consideration the whole of the evidence given above, I think that the following statements may be regarded as definitely established.

Manurial treatment affects the infestation by gout fly of summer barley by:

(1) Altering the percentage of shoots which, at the time of oviposition, are in a stage of growth favourable for oviposition.

(2) Altering the rate of early growth of stem and ear and consequently altering the number of leaf laminae which, at the time of oviposition, will arise from a shoot above the level of the apex of the ear: only eggs laid upon these leaves can give rise to the typical damage.

(3) Altering the rate of growth of the ear-bearing internode during the period of incubation of the egg, and consequently altering the number of ears which, during the incubation of the egg, will grow up above the leaf bearing the egg and so escape infection.

(4) Altering the degree of maturity (and consequently the toughness) of the ear and ear-bearing internode. This may usually be regarded as forming part of effect No. 3. As a separate effect it is very rare and may be disregarded.

Thus the entire effect of the manures upon infestation is due to their effect upon the plant growth and is not a direct effect upon the larval nutrition. The determination of this fact is of considerable importance in several ways.

(1) If the manures act directly upon the nutrition of the larva they should act in the same way in all years and in all parts of the country. As, however, their action is upon the growth of the plant their effect may be expected to vary under different climatic conditions and upon different types of soil. This is in fact the case.

(2) If the manures act directly upon the larva they might be expected to have similar effects on other Acalyptrate larvae (and quite possibly on the majority of insect larvae) feeding on and living within the shoots or leaves of cereals. Since they act only by their action on plant growth this generalisation cannot be made. Each pest must be considered separately with regard to its interrelation with the growth of the plant: only then can be estimated the possible effect of a particular manure whose effect upon plant growth is known.

(3) If the manures had been found to be acting upon the larva (*e.g.* by altering the constitution of the cell sap of the plant in such a way as to lower its feeding value for the larva or possibly making it even injurious to the larva) this would have opened up the possibility of dressing the soil with substances other than those usually used as manures. It might have been possible to discover a substance which could be absorbed in small quantities without injury to the plant, but which would, directly or indirectly, alter the constitution of the cell sap in such a way as to render it harmful to the larva. Injury to the plant would, of course, only be prevented if the larva was killed at an extremely early stage, but the repeated use of such a substance would end in the almost complete eradication of the gout fly. I am indebted to Dr Davidson for permission to refer to some recent and as yet unpublished work of his upon *Aphis rumicis* which proves that, for this insect at all events, substances added to the soil in which the host plant is growing can affect the infestation numbers. The efficiency of different manures in reducing infestation by gout fly being entirely bound up with their efficiency in promoting early plant growth, the utility of searching for a soil dressing such as that mentioned above becomes negligible.

THE EFFECT OF LATE DRESSINGS WITH NITROGENOUS MANURE.

A dressing of nitrogenous manure when the plants show signs of attack has several times been recommended as a remedial measure for plants attacked by the summer generation of *Chlorops taeniopus*. I have no hesitation in saying that when once a larva is established in a shoot no remedial measures are possible. Nothing will cause growth to recommence when once it has been stopped by the larva; even the removal of the larva (unless at the very beginning of its life) will have no effect in this way. It is therefore useless to wait until signs of attack are apparent before applying a manure. The following experiment was set up to determine whether late dressings of nitrogenous manure would have any effect if applied immediately before oviposition commenced or if applied when oviposition was mainly finished. A plot of barley was marked off on Hoos Field; the plot was 18 ft. long and 10 rows of barley ran through its length. The plot was divided transversely into three and longitudinally into two, thus dividing it into six plots each 2 yards long and containing five rows of barley. Plot XP was given 31·7 gms. (= 1½ cwt. per acre) of $NaNO_3$ on May 29th and plot ZP was given the same dressing of $(NH_4)_2SO_4$; YP was given no nitrate. Plot XQ was given 31·7 gms. of $NaNO_3$ on June 14th and plot ZQ was given the same dressing of $(NH_4)_2SO_4$; YQ was unmanured. The plot was harvested on August 23rd and each row of each plot examined separately in the laboratory. The mean infestation of the five rows for any plot was taken as the infestation figure for the plot. The infestation percentages are as follows:

YP, 6·66 %; ZP, 6·13 %; XP, 2·31 %; YQ, 10 %; ZQ, 7·3 %; XQ, 6·36 %

XP is the only plot upon which marked reduction of infestation has been obtained, and I am indebted to Mr R. A. Fisher, who has examined these figures, for the statement that the reduction is quite significant. The difference of infestation between YP and YQ, which were both unmanured and should therefore have given the same figure of infestation, though rather large, is not significant.

Thus the application of $NaNO_3$ to the plant at the commencement of the period of oviposition appears to have helped on the growth of the ear and stem and so reduced infestation. The $NaNO_3$ applied on June 14th was too late to produce any effect on growth before the eggs had hatched and the larvae penetrated into the plant. The $(NH_4)_2SO_4$ dressing had no effect either as an early or a late dressing. Mr Fisher suggests that this may be due to delayed action on the part of this manure owing to the time taken for its nitrification. If so an earlier application of the $(NH_4)_2SO_4$ would have caused a reduction in infestation.

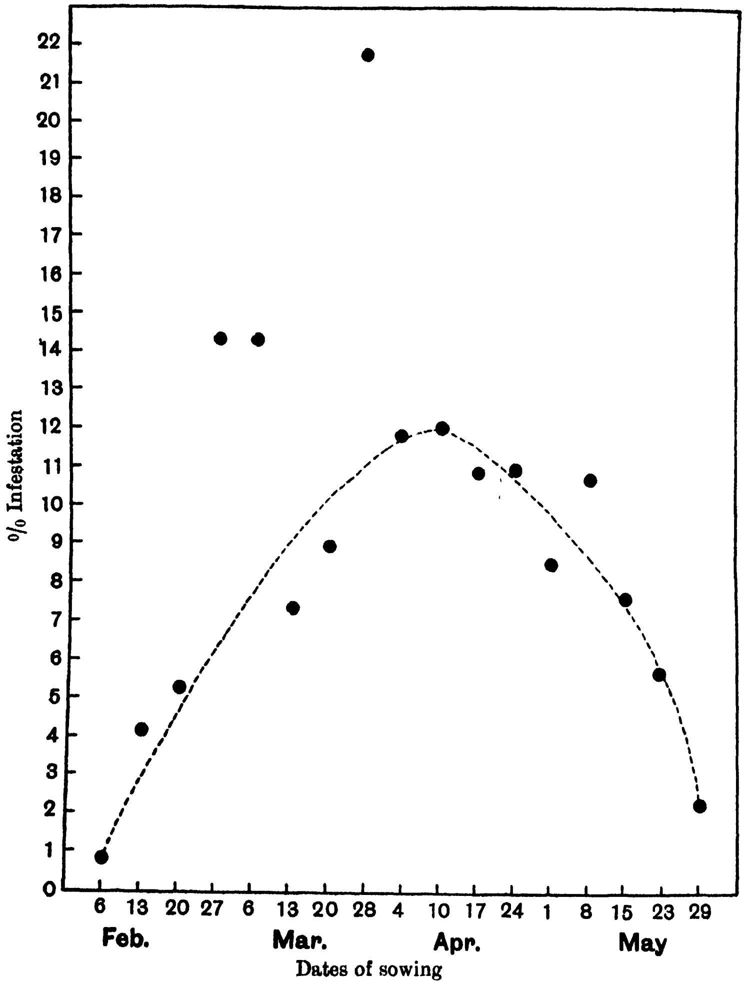
In spite of this experimental result late dressings of nitrogenous manures cannot be recommended as preventative measures against gout fly attack. The area upon which this experiment was carried out is a part of Hoos Field, outside the experimental plots, which carries barley every year but which has for many years received no manure. The land is therefore impoverished. In ordinary farm practice barley would not be grown upon such poor land and to give such a dressing as was used in this experiment might result in giving the plants an overdose of nitrogenous manures. As is shown on some of the plots on Hoos Field, repeated heavy dressings of nitrogenous manures have no beneficial effect in reducing infestation and may even have an adverse effect. I am indebted to Dr W. E. Brenchley for some interesting notes concerning the effect upon the growth of barley of varying quantities of nitrogenous manures. These notes show that while a small quantity of nitrogenous manures ($NaNO_3$ was used) may accelerate the growth of the ears, an excessive dressing has a very marked effect in retarding such growth. In districts where gout fly attack is bad, nitrogenous manures should therefore be used with extreme care; any retarding action which they may tend to show may be overcome by a liberal application of superphosphate.

RELATION OF TIME OF SOWING TO INFESTATION.

The importance of early plant growth and maturity in reducing the infestation has already been emphasised, and one might safely assume from this that the earlier spring barley is sown the less it will suffer from gout fly attack provided the soil conditions are favourable for the early sowing. The comparative immunity of early sown spring barley from attack by gout fly has, indeed, been noted by many observers.

In 1923 an attempt was made to investigate experimentally the influence of times of sowing. A plot was marked off on a part of Hoos Field (2 yards by 25 yards) and six rows of barley were sown (except the first two sowings which were five rows each) every week from Feb. 6th until May 29th. The barley was sown regardless of soil conditions which were in many cases, particularly for the early sowings, extremely bad. The soil on Hoos Field is extremely unfavourable for the success of such an experiment as this; to obtain good soil conditions in the heavy soil of this field the time of working the soil is of the greatest importance, and has to be chosen with great care. Obviously no attention is paid to this when sowings of barley are made on fixed dates. The actual percentages of infection for the various dates of sowing cannot therefore be considered as really accurate. They do, however, show definitely the relation of infestation to time of sowing. Far better figures would probably be obtained by repeating the experiment on a lighter soil which would give a greater degree of uniformity of soil conditions throughout the experiment.

The plot was harvested on August 22nd, the plants being uprooted instead of being cut so that all shoots were obtained. Each row was examined separately in the laboratory, the mean infestation figure for the six rows planted at each time of sowing being thus obtained. The results are shown graphically in Fig. 3. Fourteen of the seventeen means



Text-fig. 3. Curve showing the correlation of times of sowing of spring barley and its infestation by gout fly.

fall fairly close to the smooth curve shown on the graph. The infestations are very low for the early sowing, attain a maximum for the rows sown on April 10th and then fall off, slowly at first but more rapidly for rows sown after May 8th. This is precisely the type of curve which is expected. The early sown plants are not heavily infected because they are too well grown at the time of oviposition. The very late sown plants (those sown after May 8th) did not germinate until the end of May and beginning of June. By the time they had made sufficient growth to be suitable for oviposition there were very few *Chlorops* about. Such late sowing as this is, of course, impossible in practice as the barley would not ripen except in an extremely exceptional year.

The infestations of the rows sown on Feb. 27th and March 6th do not fall on the smooth curve and are exceptionally high. The high infestation is probably due to a caking of the soil surface which was noticed at the beginning of April to be particularly bad on these twelve rows. This, of course, hindered plant growth very seriously. The cultural conditions were bad for all the first six sowings but the two mentioned above were probably those which were most seriously affected.

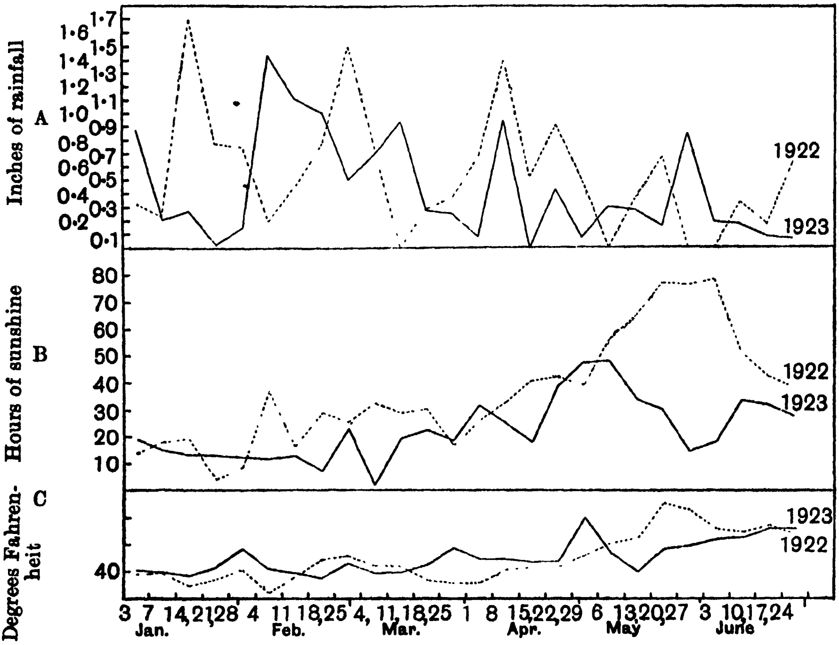
Rows 41–46 (sown March 28th) also show an abnormally high figure of infestation. I can find nothing to account for the exceptionally poor growth upon these six rows but they were undoubtedly very poor. Rows 41–46 gave, at harvest, only 46 barley shoots compared with 112 on rows 35–40 and 196 on rows 47–52. Rows 41–46 were therefore suffering from adverse cultural conditions in some way and hence the high figure of infestation.

The experiment shows therefore that time of sowing of barley has a marked effect upon its infestation by gout fly but the experiment needs repeating upon a more favourable type of soil and it is hoped that this will be carried out in 1924.

COMPARISON OF 1921-22 AND 1922-23 IN REGARD TO GOUT FLY.

Some of the most useful information that can be obtained with regard to any plant pest is that which enables one to correlate the abundance of the pest in any year with the meteorological conditions of that year and the preceding year. The ability to predict the probable infestation in any year would give a sound basis for recommending what preventative or remedial measures may profitably be undertaken. Such correlation obviously cannot be made upon only two years' observations, but a few probable correlations may be pointed out. The following observations refer almost exclusively to the summer generation which is by far the more important of the two as regards the damage done, and regarding which I have a far greater mass of observations than I have concerning the winter generation.

It is obvious in the first place that good or bad conditions for plant growth during April and May will cause respectively less or greater infestation by gout fly. The conditions for plant growth during this period were probably slightly better in 1922 than in 1923, yet comparing



Text-fig. 4. Chart showing weather conditions for the first six months of 1922 and 1923. A and B, the weekly totals of daily readings. C, the weekly means of daily maximum and minimum air temperatures. (The 1923 graphs are shown by a firm ine, those for 1922 by a dotted line.)

the infestation upon the Hoos Field plots for the two years one finds that in the majority of cases the infestation was higher in 1922 than in 1923. The reason is almost certainly as follows. In 1923 the flies emerged slightly later in spring than they did in 1922. In 1922 the weather at the end of May and beginning of June (*i.e.* the period of oviposition) was bright and sunny and the temperature was high. Oviposition was consequently stimulated and eggs were laid rapidly and prolifically. In 1923 the corresponding period was very dull and the temperature was considerably lower than in 1922; from oviposition experiments done at this time it is certain that under these conditions the flies lay very few eggs. Plant growth would undoubtedly be slowed down to some extent by the cold dull weather but would probably not be slowed down very seriously, and the plants would in many cases grow beyond the stage favourable for oviposition before the flies had laid upon them. It was probably because of this that in 1923 a considerable proportion of the flies utilised couch grass as the summer host instead of barley; in 1922 practically the whole of the summer generation was upon barley.

The flies emerged a few days later in the spring in 1923 than in 1922; if adverse conditions during the previous winter can delay the emergence of the flies for an appreciable time (*i.e.* a fortnight or more) this would almost certainly result in a very much smaller infestation of the spring barley of the following year because, presuming the spring conditions to be favourable, the plants would make good growth before oviposition commenced. Whether a hard winter would affect the winter larva in such a way as to retard the emergence of the flies I am unable to say as the winters 1921–22 and 1922–23 were both mild ones.

Comparing the summer attack on barley in 1922 and 1923 a very distinct difference in the type of damage was noticeable. In 1922 a large proportion of the attacked shoots showed the winter type of damage, and many of the others were of non-typical summer type. In 1923 the majority of the attacked shoots were of the typical form. This means that, owing to the delayed oviposition of the flies in 1923 on account of the dull weather, the plants were on the average more advanced in growth when oviposition did occur than they were in 1922. This is further shown by the relatively large number of cases in 1923 where the larva only damaged the internode.

As already mentioned, very little damage caused by the summer generation has been seen on wheat. In 1922 the wheat on New Zealand Field was damaged to the extent of possibly 3 or 4 per cent. (counts were not made) and this is the only case where the damage has been found on wheat. The reason is that wheat is an autumn sown crop (in this district) and is consequently in an advanced stage of growth in early June when oviposition occurs. The same applies to winter barley. It must not be inferred, because wheat and winter barley are not attacked by the summer generation, that the plants are inherently less susceptible to attack by gout fly than is spring barley; they may be so but their immunity is more probably due to their advanced stage of growth at the time of oviposition. In the case of New Zealand Field mentioned above the wheat had made extremely poor growth and was the most backward wheat on the whole farm.

It has been suggested by many writers (see (3) for full details) that an autumn generation of *Chlorops* may occur. Nowicki (3) considers that this may occur in certain conditions, *i.e.* when a favourable summer has led to the emergence of flies very early in autumn. As already mentioned I did not succeed in rearing an autumn generation from flies emerging early in August 1922. It cannot, of course, be deduced from this that an autumn generation never occurs, but if it does occur I imagine that even in the most favourable conditions (*e.g.* the summer of 1921) it would be only a partial generation due to a few exceptionally early emerging flies.

The probable influence of weather may then be summed up as follows. A cold winter may retard emergence of the flies in the following spring and so allow the barley to reach an advanced stage of growth before oviposition occurs. Good growing weather during April and May will reduce infestation for the same reason. Dull cold weather during the end of May and beginning of June is more disadvantageous to the flies than to the plants and will reduce infestation. A hot summer may favour the development of a partial intermediate (autumn) generation.

PARASITISATION OF *CHLOROPS TAENIOPUS.*

Up to the present observations have been made upon four Hymenopterous parasites of *Chlorops taeniopus*, and I hope shortly to deal with these in some detail in a separate paper. In this place I shall only mention briefly the effect upon the parasitisation of *Chlorops* of different times of sowing of the host plant and different manurial treatments. Upon the latter point I have extensive data drawn from the 1923 examination of plots 1AA, 1A, 6_1, 5A, 4C, 2.O. and 7_2.

Of the four parasites two—*Coelenius niger* (a Braconid) and *Pteromalus micans* (a Chalcid)—are endoparasites of the *Chlorops* larva while two others (not yet identified) are ectoparasites. The two former have been found in both winter and summer generations but the two latter have so far been found only in the summer generation. The degree of parasitisation of the *Chlorops* larvae taken from each of these plots mentioned above has been obtained for each of the four parasites and the results are extremely interesting. Here it will be sufficient to mention the total parasitisation only. This is as follows:

1AA, 18 %; 1A, 25·19 %; 6_1, 43·93 %; 5A, 14·47 %; 4C, 9·76 %; 2.O., 7·7 %; 7_2, 10·8 %

The details of the causes of this variation in parasitisation cannot be given here. Briefly the variation is caused by the fact that plots such as 4C, 2. O. and 7_2 are utilised mainly for the earliest oviposition. The imagines of the parasites emerge from their winter generation later than the *Chlorops* imagines and the endoparasites only attack the very young stages of the *Chlorops* larvae. The very early *Chlorops* larvae therefore escape parasitisation by the endoparasites; there was not a single endoparasite in the 90 larvae examined from plot 2. O. and plot 7_2. The ectoparasites, as might be expected, attack a later and more mature stage of the *Chlorops* larva and the early *Chlorops* larvae do not therefore escape parasitisation by these.

The effect of time of sowing upon parasitisation is only known by analogy with the above results. The infected shoots on the experimental plot set up to determine the effect of different times of sowing upon infestation of the plants by gout fly, could not be examined for parasitisation as it was necessary for the purpose of the experiment to leave the plants growing until harvest time. It is fairly certain that the larvae in very early sown plants would be little parasitised and that parasitisation would increase in the later sowing.

The same type of result is obtained for the winter generation of *Chlorops* except that very late *Chlorops* larvae which are sometimes found (see above, p. 190) are not parasitised at all because the imagines of the two endoparasites which attack the winter generation do not live on after cold weather has set in. The *Chlorops* imagines themselves are much less susceptible to cold. The parasitisation of the winter generation of 1922–23 was in general very high, averaging about 40 per cent.

These results demonstrate that all measures taken for the promotion of early plant growth will have the unfortunate effect of reducing the parasitisation of the summer generation. One would greatly have preferred finding the reverse effect. It is difficult to estimate the amount of importance which should be attached to this effect upon the parasites. Probably it is not so serious as the above figures suggest. If by the promotion of rapid early growth spring barley crops are rendered unsuitable as host plants for *Chlorops*, couch grass will be utilised instead. This happened to quite a considerable extent in 1923 (owing rather to the delayed oviposition of the flies than to good early growth of the plants). I did not examine infected couch grass shoots in the summer of 1923, but it is probably safe to assume that the larvae in such shoots would be parasitised to a normal extent. The couch grass would therefore tend to stabilise the balance of *Chlorops* with its parasites. It might even tend to increase the percentage of parasitisation because a large part of the attacked couch grass would be on root fields which are constantly cultivated during the early stages of growth of the roots; many of the early larvae would be destroyed owing to the death of their hosts due to this cultivation. The later larvae would not be destroyed to quite the same extent and it is the later larvae which are most heavily parasitised. This is rather speculative but I hope to obtain some observations on these points in 1924[1].

PREVENTIVE MEASURES.

General. The eradication of couch grass would have a very great effect in reducing the total numbers of gout flies. Complete eradication of couch grass is, of course, impossible, but it can be kept under control on arable fields and particularly on root fields where it is, apparently, most liable to attack.

Summer generation. This is the generation which does the greatest damage. The most effective measure to guard against attack is very early sowing of the spring barley. In heavy cold soil sowing cannot usually be done very early but depends, far more than is the case with light warm soils, upon weather conditions. Very early sowing on heavy cold land would probably cause far more damage to the crop by poor cultural conditions than would have been caused by the gout fly had the crop been sown later. Here, then, very early (*i.e.* February or early March) sowing is impossible though the sowing should be made at the very earliest date permissible with regard to soil conditions. It is upon soils of this type that superphosphate is particularly useful as a manure, as it stimulates rapid growth and early maturity of the ear and ear-bearing internode.

Any manure which will stimulate the early growth of the plant and tend to force up the ear and ear-bearing internode will have a beneficial effect in reducing infestations. It is by no means certain that artificial manures always react in the same way upon plant growth; their action may vary with variations of soil type and climatic conditions. Superphosphate seems to be remarkably constant in its action in promoting early maturity and the use of this manure is almost certain to have beneficial results in reducing gout fly infestation. Farmyard manure seems also to be uniformly beneficial. The necessity for caution in applying nitrogenous manures has already been indicated. With regard to other artificial manures no general recommendation can be made. Thus in 1922, K_2SO_4 had a very marked effect in reducing infestation, but this effect was not repeated in 1923. These points will be found in greater detail in the analysis of the manurial counts by "Mathetes" (5).

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[1] Some data concerning the parasitisation of the larvae of the summer generation of *Chlorops* on couch grass had been overlooked when the above was written. Three separate counts were done from July 18th to July 21st, 1923; in all 191 *Chlorops* larvae were examined, and the figures tend to confirm the speculations given above. The *Chlorops* larvae were parasitised to the extent of 41%—the endoparasites being markedly predominant.

Good cultural conditions of the soil are naturally of great importance as they have a great effect upon plant growth. It is because of the poorer cultural conditions that the headlands of a field of barley are often more heavily attacked by gout fly than the rest of the field.

Arthur (*Gardener's Chronicle*, 1844, p. 365) recommends the application to the land of noxious perfumes "such as soot, guano, or sand immersed in gas water" to prevent the fly alighting on the fields. I can offer no opinion as to whether this would be efficacious or not and the recommendation does not appear to be based upon any definite observation or experiment.

Winter generation. The damage done to winter barley and winter wheat crops is, so far as I have seen, quite negligible. This is not always the case; Jablonowski gives the following figures (quoted in (2)) for the winter 1909–1910. Wheat, 34·18 per cent. attacked; Rye[1], 60·10 per cent. attacked. Late sowing of autumn crops will minimise the attack, but the sowing must not be so late as to retard the growth in the following spring or the plants may be attacked by the summer generation. Fulmek (2) gives a very clear account of the points to be considered in connection with the winter generation. The actual damage done by the winter generation is probably not great as the shoots killed will be replaced by fresh tillers which will develop sufficiently early to bear good grain. Nevertheless if the winter attack is disregarded and the seed sown early in autumn a plentiful supply of winter hosts is being provided and the spring barley of the next year is liable to suffer badly. It is therefore advisable to sow winter wheat and barley as late in the autumn as can be done with safety, having regard to growth conditions.

The danger of winter hosts other than couch grass and winter cereals has already been indicated and means of prevention given (pp. 189–190).

SUMMARY.

Chlorops taeniopus passes through two generations per year. The winter generation is mainly upon couch grass but also occasionally upon winter wheat or barley or upon self-sown wheat or barley. The summer generation is mainly upon spring barley but in seasons unfavourable to the fly couch grass may be utilised. Very rarely wheat is a summer host plant. The life-history is described in detail.

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[1] Rye is not grown in the Harpenden district and I have no observations concerning the attack of gout fly upon rye. It does occur in this country.

The type of distortion caused to the host plant depends on the stage of growth of the plant when attacked and the degree of distortion of the plant depends upon the rate of growth at the time of attack.

The relation of the fly to the different kinds of host plants is described particularly as regards the winter generation, and is shown to vary with such factors as date of emergence of the flies, weather conditions during the oviposition period and amount of growth of the different kinds of host plants.

In dull and cool weather the flies will lay few eggs but are stimulated to rapid egg laying by bright and sunny weather. A single fly may lay about 150 eggs. More than one act of coitus is necessary to fertilise all the eggs which a ♀ is capable of laying. The length of life of the imagines is probably about a fortnight for flies emerging in spring but may be over two months for the autumn emerging flies.

Certain manures (particularly superphosphate) are shown to have a marked beneficial effect in reducing the infestation of summer barley by gout fly. The effect of the manures is shown to be due entirely to their stimulating effect upon the maturing of the ear and the growth of the ear-bearing internode.

It is shown that while small dressings of nitrogenous manures may reduce infestation, large dressings will not reduce it and may have a tendency to retard growth of the ear and so increase infestation.

Early sowing of spring barley is shown to be efficacious in preventing infestation by gout fly.

Preventative measures suggested are early sowing of spring barley, good cultural conditions of the soil, and manuring (*e.g.* with superphosphate or farmyard manure) to stimulate early growth.

LITERATURE.

(1) FREW, J. G. H. (1923). On the larval anatomy of *Chlorops taeniopus* Meig. and two related Acalyptrate Muscids, with notes on their winter host plants. (*P.Z.S.* p. 783.)

(2) FULMEK, L. (1911). Zum Auftreten der Halmfliege (*Chlorops taeniopus* Meig.) in Weizen. (*Osterreichischen Agrar-Zeitung* Nr. 30 vom 29 Juli.)

(3) NOWICKI, M. (1871). Ueber die Weizenverwusterin *Chlorops taeniopus* Meig. und die Mittel zu ihrer Bekampfung. (*Verh. Zoologischbotanischen Gesellschaft in Wien.*)

(4) ORMEROD, E. A. (1890). Manual of Injurious Insects and Methods of Prevention (2nd ed. London, pp. 75–79).

(5) "MATHETES" (1924). Statistical study on the effect of manuring on infestation of barley by Gout Fly. (*Ann. App. Biol.* XI, 2.)

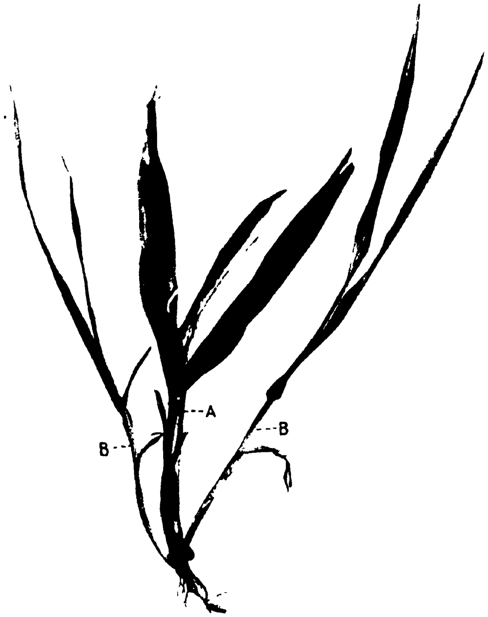

Fig 1

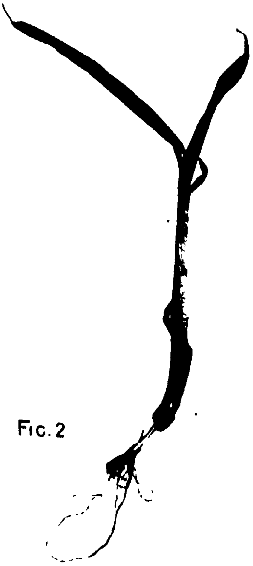

Fig. 2

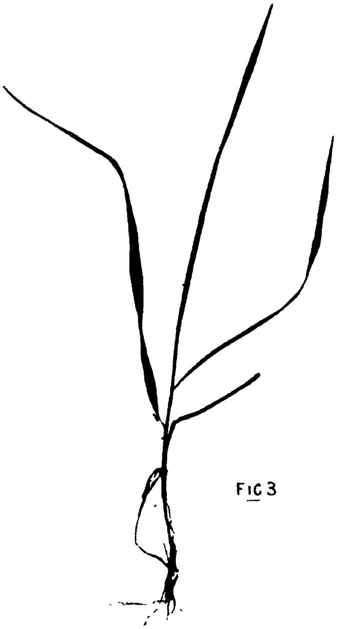

Fig 3

FREW.—On *Chlorops taeniopus* Meig. (The Gout Fly of Barley). (pp. 175—219.)

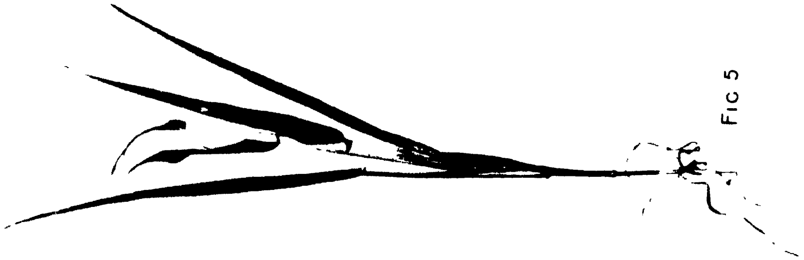

Fig 5

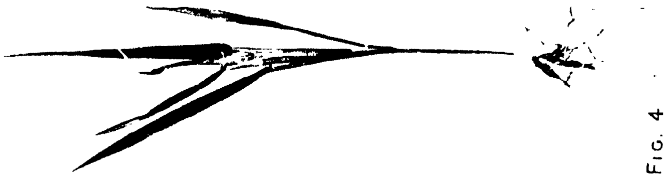

Fig. 4

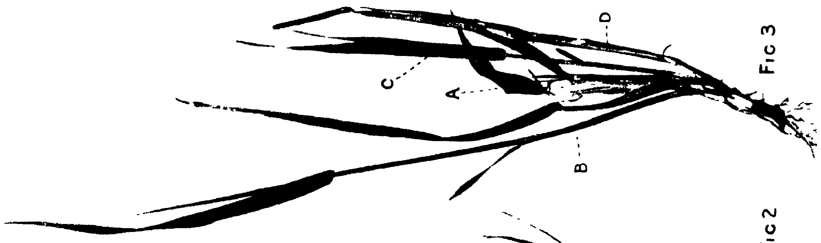

Fig 3

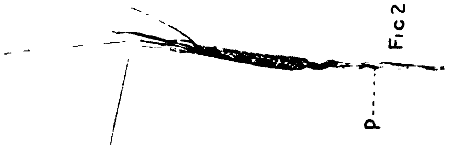

Fig 2

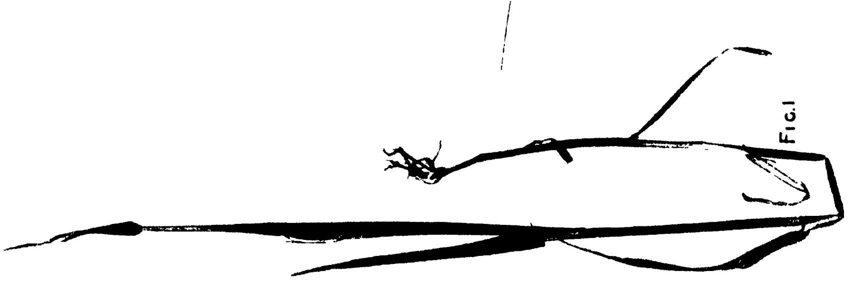

Fig. 1

FREW.—On *Chlorops taeniopus* Meig. (The Gout Fly of Barley). (pp. 175—219.)

EXPLANATION OF PLATES III AND IV

(For the photographs here reproduced I am indebted to Mr H. M. Morris.)

Plate III.

Fig. 1. A plant of winter barley showing the appearance of a shoot *A* attacked by gout fly compared with two normal shoots *B*.

Fig. 2. A shoot of couch grass attacked by the winter larva of *Chlorops taeniopus*.

Fig. 3. A normal shoot of couch grass during the winter.

Plate IV.

Fig. 1. The typical form of summer attack on barley.

Fig. 2. Ear and ear-bearing internode from a typical example of barley attacked by the summer generation of *Chlorops taeniopus*. *P* the puparium. Pupation has taken place in the food groove below the ear.

Fig. 3. Summer damage on barley resembling the winter form. *A* the attacked shoot; *B*, *C* and *D* unattacked shoots. *D* is a normal ear-bearing shoot which has been broken short.

Figs. 4 and 5. Non-typical forms of summer damage on barley.

(*Received September 27th*, 1923.)

STATISTICAL STUDY ON THE EFFECT OF MANURING ON INFESTATION OF BARLEY BY GOUT FLY

By "MATHETES."

(From the Statistical Department, Rothamsted Experiment Station.)

THE material supplied for this investigation consisted of the numbers of shoots injured in each of 10 samples of 100 taken from differently manured plots in various fields during 1922 as described in (4): the number of plots sampled is set out below, and the details are given in Appendix I.

Permanent Fields:	
Hoos Field	28
Woburn Stackyard Field	22
Malting Barley Experimental Fields:	
Long Hoos	7
Woburn	5
Dunmow (Mr Hasler's Farm)	5
Wellingore (Mr Nevil's Farm) ...	5

In order to determine whether there are significant differences between plots, corresponding to different manurial conditions, it was necessary first to ascertain whether the distribution of attack within the plots was random, in which case the mean infestation of any plot (see Appendix I) could be taken with confidence as a basis for comparison with other plots. Accordingly a preliminary survey was made along the lines developed successfully by Fisher (1) in determining the accuracy of the plating method of estimating the density of bacterial populations in the soil. If the numbers in the sub-samples be represented by

$$x_1, x_2, x_3, \dots x_n,$$

so that $S(x) = n\bar{x}$, the index of dispersion

$$\chi^2 = \frac{S(x-\bar{x})^2}{\bar{x}}$$

can be calculated for each plot and if the samples can be represented as random samples from a Poisson series the distribution of the χ^2's should give a good fit when compared with the expected distribution taken from Elderton's tables entered with $n' = n$, the distribution of χ^2 being independent of $\bar{x}$.

In the present case we wish to test if the distribution agrees, not with the Poisson, but with the binomial distribution: this may be done with exactitude by modifying the formula for the index of dispersion. In such a case the formula for χ^2 becomes

$$\chi^2 = \frac{NS(\xi)^2}{(N-\bar{x})\bar{x}} = \frac{S(\xi)^2}{N\bar{p}\bar{q}},$$

where N is the number in the sub-sample (here = 100).

$$\xi = x - \bar{x}; \quad N\bar{p} = \bar{x};$$

$\bar{p}$ is the probability of attack and $\bar{q}$ of non-attack. The values of χ^2 were calculated from the above formula, and the correlation between mean infestation and χ^2 was found to be insignificant. The fit over the whole distribution was not very good, as the following table shows.

Table I.

The distribution of χ^2 for 70 plots compared with the expected distribution.

χ^2	Expected	Realised	Difference	d^2/E
< 3	2·52	1	$-1·52$	·9168
$> 3 < 5$	9·10	4	$-5·10$	2·8582
$> 5 < 7$	13·79	6	$-7·79$	4·4006
$> 7 < 9$	14·00	14	$\pm 0·00$	—
$> 9 < 11$	11·27	12	$+ ·73$	·0473
$> 11 < 13$	7·91	11	$+3·09$	1·2071
$> 13 < 15$	5·04	9	$+3·96$	3·1115
$> 15 < 17$	2·94	3	$+ ·06$	·0012
$> 17 < 19$	1·68	4	$+2·32$	3·2038
> 19	1·75	6	$+4·25$	10·3217

$$\chi^2 = 26·0682$$
$$P = ·002$$

The ordinary test for goodness of fit shows the observed distribution to be more widely divergent from the expected than would be accounted for by the errors of random sampling. A more delicate test in such a case is to compare the total χ^2 with the calculated value, using the approximate formula $\sqrt{2n'-3}$ to give P for values of n' outside the range of Elderton's tables. Here we find

$$S(\chi^2) = 790·99$$
$$\sqrt{2\chi^2} = 39·773$$
$$\sqrt{2n'-3} = 35·548$$

$$\text{Difference} = 4·225$$

This difference corresponds to the x/σ of the probability integral tables and being greater than 2 is definitely significant. The distribution cannot, therefore, strictly speaking be regarded as consisting of random samples from a binomial series.

For individual fields we get the results shown in Table II.

Table II.

	Hoos		Woburn Stackyard		Long Hoos		Woburn		Dunmow		Wellingore	
χ^2	Expected	Realised	Expected	Realised	Expected	Realised	Expected	Realised	Expected	Realised	Expected	Realised
< 3	1·008	—	·720	1	·252	—	·180	—	·180	—	·180	—
$> 3 < 5$	3·640	—	2·600	—	·910	2	·650	1	·650	—	·650	1
$> 5 < 7$	5·516	1	3·940	5	1·379	—	·985	—	·985	—	·985	—
$> 7 < 9$	5·600	5	4·000	3	1·400	1	1·000	2	1·000	1	1·000	2
$> 9 < 11$	4·508	5	3·220	4	1·127	—	·805	1	·805	2	·805	—
$> 11 < 13$	3·164	6	2·260	4	·791	1	·565	—	·565	—	·565	—
$> 13 < 15$	2·016	4	1·440	1	·504	1	·360	—	·360	1	·360	2
$> 15 < 17$	1·176	1	·840	—	·294	1	·210	1	·210	—	·210	—
$> 17 < 19$	·672	2	·480	1	·168	—	·120	—	·120	1	·120	—
> 19	·700	4	·500	1	·175	1	·125	—	·125	—	·125	—

which may be conveniently summarised thus:

Table III.

	Hoos		Woburn		Malting Barleys	
χ^2	Expected	Realised	Expected	Realised	Expected	Realised
< 3	10	1	7	6	8	4
$> 7 < 15$	15	20	11	12	12	14
> 15	3	7	2	2	2	4

In the case of Woburn Field and the Malting Barleys, the fit is sufficiently good to enable us to conclude that the sampling is random. In Hoos, however, the fit is anything but good, showing the intensity of attack to have varied within the plot more than is consistent with random sampling.

To test whether there were significant differences between plots, the distribution of χ^2 with $N = 1000$ and $\xi =$ mean of plot minus mean of field, compared with the expected value was examined. For instance, in the case of Woburn

$$\chi^2 = \frac{S(x - \bar{x})}{N\bar{p}\bar{q}} = 193{\cdot}9,$$

$n' = 20$, so that there were significant differences between plots. Such differences were found also in Hoos, Long Hoos and Wellingore, as the following table shows:

Table IV.

Expected and Realised Values of χ^2 over whole fields.

	χ^2	n'	Difference between plots
Hoos	548·043	28	Significant
Woburn	123·353 193·908	18 } * 20 }	do. do.
Malting Barleys:			
Long Hoos	13·889	7	Significant
Woburn	5·359	5	Not significant
Dunmow	5·966	5	do.
Wellingore	30·093	5	Significant

* Two of the Woburn plots were very badly over-grown with weeds; the comparison is shown with and without these plots.

An interesting point in connection with Woburn Stackyard Field is that there was a marked indication that excessive soil acidity reduced infestation. On the very acid plots, however, the weeds were so numerous and the barley so scarce, that the infestation figures were rather suspect. It is a point which should be susceptible to experimental treatment, and for what they are worth, particulars are given of the very acid plots in Woburn, and their parallels where the acidity is reduced by the addition of lime.

Table V.

Plot No.	Soil *p*H value	Infestation	Treatment
2A	4·15	8·2	Sulphate of Ammonia ($=25$ lbs. NH_3)
2AA	5·01	16·3	do + 5 cwts. Lime 1905, 1909, 1910, 1912
5A	4·40	13·3	do. + Minerals
5AA	5·14	17·8	do. + do. + 1 ton Lime 1905, 1916
8B	4·36	6·3	do. (alternate years) ($=50$ lbs. NH_3) + Minerals
8BB	6·61	16·9	do. + Minerals + 2 tons Lime 1897, 1912

No great importance is attached to these figures; they are merely given as being suggestive and perhaps worthy of experimental investigation.

Having found that significant differences do exist between plots, the next step was to compare plots differing in one manure, so that if a significant difference is found, it can fairly be ascribed to the presence or absence of that manure. The method of comparison was in all cases the same, viz. if we have the following scheme:

	Number attacked	Number immune	
Superphosphate	a	b	$a+b$
No superphosphate	c	d	$c+d$
	$a+c$	$b+d$	$a+b+c+d$

Fisher has shown (2) that if

$$x = \frac{bc - ad}{(a+b)(c+d)}, \text{ then } \frac{x^2}{\sigma_x^2} = \frac{(bc-ad)^2(a+b+c+d)}{(a+b)(c+d)(a+c)(b+d)} = \chi^2,$$

and that χ for $n' = 2$ is distributed over the positive half of a normal curve with unit standard deviation.

By determining $\chi = \sqrt{\frac{x^2}{\sigma_x^2}}$, we have at once a measure of significance: if $\chi > 2$, the difference is significant. As many comparisons as possible were made in this way, with the results set out below (*v.* Table VI). It should be noted here, that in the case of Hoos Field, since the mean χ^2 was about 25 per cent. higher than the expected value, the limit for significance in this case should be taken as 2·5 instead of 2·0.

The best set of comparisons is obtained from Hoos, but unfortunately very few comparisons are possible between the same pairs of manures in different fields, and even these do not to any great extent agree with the results for Hoos. This, of course, was to be expected, considering the previous history of the other fields compared with Hoos, where the plots have had the same manures for many years.

Considering the result for Hoos where any value of χ greater than 2·5 marks a significant effect of manure on infestation (+ indicates a beneficial effect), it will be seen that Superphosphate is almost invariably effective in reducing infestation, either alone or in conjunction with other manures. The only exceptions found were with Alkali Salts and Nitrate of Soda, and with the same combination + Silicate.

Farmyard Manure alone is very effective and so is Potash in presence of Superphosphate and Ammonium Salts.

Nitrate of Soda reduces infestation when acting alone, but not when used with Superphosphate, Alkali Salts or Minerals.

Ammonium Salts are only effective in conjunction with Minerals, or with Superphosphate and Potash.

Rape Cake alone has no effect, but reduces infestation when used with Superphosphate, Alkali Salts or Minerals.

Minerals either alone or with Ammonium Salts, Nitrate of Soda or Rape Cake, are beneficial; and Alkali Salts are only of service in presence of Nitrate of Soda or Rape Cake.

Hoos Field, on account of its previous history, is very valuable in any attempt to arrive at a quantitative estimate of the effects of different manures, and the remainder of the work, consisting of such an attempt, dealt entirely with Hoos. The idea was to determine for each manure

Table VI. *Values of χ for fourfold manurial comparisons.*

	Permanent Barley				
	Hoos	Woburn	Long Hoos	Wellin-gore	Long Hoos Woburn Wellingore Dunmow
Superphosphate *v.* unmanured	+3·522				
„ with Rape Cake	+5·859				
„ „ Ammonium Salts (+Potash)	+3·577	—	+1·022	−5·621	−1·076
„ „ Sodium Nitrate	+2·688	−2·364			
„ „ Alkali Salts	+3·303				
„ „ „ +Ammonium Salts	+8·787				
„ „ „ +$NaNO_3$	+1·699				
„ „ $NaNO_3$+Silicate	+3·914				
„ „ „ + „ +Alkali Salts	+2·084				
„ „ Rape Cake+Alkali Salts	+3·782				
Farmyard Manure *v.* unmanured	+7·434	+4·560			
Potash with $NaNO_3$	—	+1·317			
„ „ Superphosphate	+3·400				
„ „ „ +Ammonium Salts	+6·625	—	+2·704	−2·001	+ ·802
Sodium Nitrate *v.* unmanured	+4·139	25 lbs. NH_3 +4·491 50 lbs. NH_3 +3·465			
„ „ with Superphosphate	+1·783				
„ „ „ Alkali Salts	+2·259				
„ „ „ Minerals†	+1·354	− ·060			
Ammonium Salts *v.* unmanured	− ·463	+8·909			
„ „ with Superphosphate	+ ·066				
„ „ „ Alkali Salts	−1·490				
„ „ „ Minerals	+4·262	+5·317*			
„ „ „ Superphosphate+Potash	+3·476	—	+ ·834	−2·266	− ·992
Rape Cake *v.* unmanured	− ·332	+2·994			
„ „ with Superphosphate	+2·514				
„ „ „ Alkali Salts	+4·178				
„ „ „ Minerals	+4·642				
Minerals *v.* unmanured	+5·507	+2·994			
„ with Ammonium Salts	+9·311	−1·425*			
„ „ $NaNO_3$	+4·013				
„ „ Rape Cake	+3·019				
Alkali Salts *v.* unmanured	+1·900				
„ „ with $NaNO_3$	+2·671				
„ „ „ Ammonium Salts	+ ·574				
„ „ „ Rape Cake	+6·077				
Sodium Silicate with $NaNO_3$	−1·905				
„ „ „ „ +Alkali Salts	+ ·767				
„ „ „ „ +Superphosphate	+ ·145				
„ „ „ „ +Minerals	+1·226				
Lime with $NaNO_3$	—	25 lbs. NH_3 + ·517 50 lbs. NH_3 − ·725			
„ „ Minerals	—	−1·177			
„ „ Ammonium Sulphate	—	−5·524			

* Include plots overgrown with weeds.

† Minerals in the case of Hoos means Potash, Superphosphate, Sodium and Magnesium Sulphates. The same was true of Woburn until 1905, since when it has meant Superphosphate and Potash only.

a figure showing its efficiency in reducing infestation, and thus form an equation to predict the infestation on any plot solely from a consideration of the manurial treatment. The mathematical theory of the method employed has been developed by Fisher (3), and was applied in the following way. From the percentages immune, and the number of observations on 26 of the Hoos plots (Farmyard Manure and ashes plots omitted), weights were obtained by the method of (3), and from the 26 observational equations, seven normal equations derived, the solution of which gave the values of the seven unknowns:

q_O representing the % immunity conferred by no manures.
q_R representing the additional % immunity conferred by Rape Cake
q_A ,, ,, ,, ,, ,, Ammonium Sulphate
q_N Nitrate of Soda
q_S Sodium and Magnesium Sulphate
q_K ,, ,, ,, ,, ,, Potash
q_P ,, ,, ,, ,, ,, Superphosphate

The following table shows the arrangement of the work:

Table VII.

Plot No.	Manurial treatment	% immune	No. immune (x)	No. of observations (n)	n^2/y	n^3/xy	Suffixes involved
7.1	Unmanured after Dung	81·8	818	1000	5494·5	6717·03	O
6.1	Unmanured	80·9	809	,,	5235·6	6471·73	O
1.0	Unmanured	82·1	821	,,	5586·6	6804·48	O
2.0	Superphosphate	86·6	866	,,	7462·7	8617·18	O P
3.0	Alkali Salts	84·3	843	,,	6369·4	7555·38	O P S
4.0	Complete Minerals	89·3	893	,,	9345·8	10465·43	O P K S
5.0	Superphosphate + Potash	91·3	913	,,	11494·0	12589·38	O P K
1A	Ammonium Salts	80·8	808	,,	5208·3	6445·79	O A
2A	do. + Superphosphate	86·7	867	,,	7518·8	8672·18	O A P
3A	do. + Alkali Salts	81·8	818	,,	5494·5	6717·03	O A S K
4A	do. + Complete Minerals ...	94·5	945	,,	18182·0	19240·19	O A K P S
5A	do. + Potash + Superphosphate ...	95·2	952	,,	20833·0	21882·98	O A K P
1AA	Sodium Nitrate	80·5	805	,,	5128·2	6370·25	O N
2AA	do. + Superphosphate ...	89·2	892	,,	9259·3	10380·60	O N P
3AA	do. + Alkali Salts	87·8	878	,,	8196·7	9336·04	O N K S
4AA	do. + Complete Minerals ...	91·1	911	,,	11236·0	12333·76	O N K P S
1AAS	do. + Sodium Silicate	83·4	834	,,	6024·1	7222·90	O N
2AAS	do. + do. + Superphosphate ...	89·4	894	,,	9434·0	10552·87	O N P
3AAS	do. + do. + Alkali Salts ...	88·9	889	,,	9090·9	10226·35	O N K S
4AAS	do. + do. + Complete Minerals	92·6	926	,,	13514·0	14593·77	O N K P S
1C	Rape Cake	81·0	810	,,	5263·2	6497·95	O R
2C	do. + Superphosphate	90·2	902	,,	10204·0	11312·15	O R P
3C	do. + Alkali Salts	90·5	905	,,	10526·0	11631·23	O R K S
4C	do. + Complete Minerals ...	94·9	949	,,	19608·0	20660·95	O R K P S
1N	Sodium Nitrate	87·0	870	,,	7692·3	8841·53	O N
2N	Sodium Nitrate	90·1	901	,,	10101·0	11211·10	O N

From this we derive the following Normal Equations:

$$\begin{aligned}
273350{\cdot}23\,q_O + 168856{\cdot}82\,q_P + 149677{\cdot}11\,q_K + 122760{\cdot}13\,q_S + 101069{\cdot}17\,q_N + 62958{\cdot}17\,q_A + 50102{\cdot}28\,q_R &= 243502{\cdot}9\\
168856{\cdot}82\,q_O + 168856{\cdot}82\,q_P + 111766{\cdot}46\,q_K + 84849{\cdot}48\,q_S + 47861{\cdot}00\,q_N + 49795{\cdot}35\,q_A + 31973{\cdot}10\,q_R &= 154461{\cdot}0\\
149677{\cdot}11\,q_O + 111766{\cdot}46\,q_P + 149677{\cdot}11\,q_K + 115204{\cdot}75\,q_S + 46489{\cdot}92\,q_N + 47840{\cdot}20\,q_A + 32292{\cdot}18\,q_R &= 137520{\cdot}9\\
122760{\cdot}13\,q_O + 84849{\cdot}48\,q_P + 115204{\cdot}75\,q_K + 122760{\cdot}13\,q_S + 46489{\cdot}92\,q_N + 25957{\cdot}22\,q_A + 32292{\cdot}18\,q_R &= 111563{\cdot}3\\
101069{\cdot}17\,q_O + 47861{\cdot}00\,q_P + 46489{\cdot}92\,q_K + 46489{\cdot}92\,q_S + 101069{\cdot}17\,q_N + \text{—} + \text{—} &= 89676{\cdot}5\\
62958{\cdot}17\,q_O + 49795{\cdot}35\,q_P + 47840{\cdot}20\,q_K + 25957{\cdot}22\,q_S + \text{—} + 62958{\cdot}17\,q_A + \text{—} &= 57236{\cdot}6\\
50102{\cdot}28\,q_O + 31973{\cdot}10\,q_P + 32292{\cdot}18\,q_K + 32292{\cdot}18\,q_S + \text{—} + \text{—} + 50102{\cdot}28\,q_R &= 45601{\cdot}2
\end{aligned}$$

The solution of the normal equations yielded the values

$$q_O = \cdot 811000 \qquad q_S = -\cdot 023727$$
$$q_R = \cdot 042208 \qquad q_K = \cdot 061439$$
$$q_A = \cdot 020737 \qquad q_P = \cdot 051159$$
$$q_N = \cdot 034363$$

whence we can place the manures in the order of their efficiency in decreasing infestation as follows:

Complete Minerals	8·9 %
Potash	6·1
Superphosphate	5·1
Rape Cake	4·2
Alkali Salts (Potash + Sodium and Magnesium Sulphates)	3·8
Nitrate of Soda	3·4
Ammonium Salts	2·1
Sodium and Magnesium Sulphates	−2·3

Still following the method of (3), the percentage immunity was calculated for each plot from these values, and the differences between the actual and calculated values compared with the standard error, with the following results:

Table VIII.

Actual	Calculated	Difference (d)	Standard error (σ)	d^2/σ^2
81·8	81·1	+ ·7	1·2	·34
80·9	81·1	− 1·1	1·2	·84
82·1	81·1	+ 1·0	1·2	·69
86·6	86·2	+ ·4	1·1	·13
84·3	84·9	− ·6	1·1	·30
89·3	90·0	− ·7	·9	·60
91·3	92·3	− 1·0	·8	1·56
80·8	83·2	− 2·4	1·2	4·00
86·7	88·3	− 1·6	1·0	2·56
81·8	87·0	− 5·2	1·1	22·30
94·5	92·1	+ 2·4	·9	7·12
95·2	94·4	+ ·8	·7	1·31
80·5	84·5	− 4·0	1·1	13·24
89·2	89·6	− ·4	1·0	·16
87·8	88·3	− ·5	1·0	·25
91·1	90·0	+ 1·1	·9	1·49
83·4	84·5	− 1·1	1·1	1·00
89·4	89·6	− ·2	1·0	·04
88·9	88·3	+ ·6	1·0	·36
92·6	93·4	− ·8	·8	1·00
81·0	85·3	− 4·3	1·1	15·28
90·2	90·4	− ·2	·9	·05
90·5	89·1	+ 1·4	1·0	1·96
94·9	94·2	+ ·7	·7	1·00
87·0	84·5	+ 2·5	1·1	5·17
90·1	84·5	+ 5·6	1·1	25·09

$$\chi^2 = 107{\cdot}84.$$

It will be seen that the majority of the results show good agreement; in only six plots was there a very wide divergence of observed and calculated, three of which were Nitrate plots alone, and one Rape Cake alone. It may be noted in passing that the Nitrate plots were so discordant that no theory could cover them. The value of χ^2 from d^2/σ^2 was 107·84 (mostly accounted for by the six bad plots) and comparing this with the value of χ^2 for the whole field, having regard to the number of degrees of freedom used, we have

$$\frac{107{\cdot}84}{19} : \frac{548{\cdot}04}{27} = 5{\cdot}66 : 20{\cdot}30,$$

and allowing $\chi^2 = 1$ for each, this reduces to 4·66 : 19·3. That is to say, we have accounted for 76 per cent. of the variance on the basis of a linear arrangement of the manures as regards their efficiency in reducing infestation; of the remaining 24 per cent., 6½ per cent. is ascribable to sampling; and considering that the 107·84 was mainly due to six exceptional plots, this result may be regarded as satisfactory, and furnishing fairly conclusive evidence that manures have a differential and quantitatively measurable effect in reducing gout fly infestation of barley.

As to whether we have been measuring the effect of the manures in conferring immunity on the plant by

(1) transference of some of its constituents into the plant, and so
  (*a*) causing a hardening of the plant tissues so as to render the passage of the larvae more difficult, or
  (*b*) rendering the plant tissues unpalatable, or

(2) by promoting early growth, it was impossible to say without further investigation, and this has now been carried out by Mr Frew who has discussed the whole question in (4).

For the purposes of this paper, it is only necessary to discuss confirmatory work carried out during 1923.

INFESTATION OF BARLEY IN HOOS FIELD, 1923.

A series of counts similar to that of 1922 was carried out in 1923, but with a few additional particulars, such as estimates of shoot density, etc. (*v.* Appendix II) and as it is of some interest to know how far the later results confirm the earlier, a brief survey is appended.

The observed and expected distribution of χ^2 is set out in Table IX.

Table IX. *Distribution of χ^2 for Hoos Field* 1923.

χ^2	Expected	Observed
< 3	1·008	1
$> 3 < 5$	3·640	4
$> 5 < 7$	5·516	5
$> 7 < 9$	5·600	3
$> 9 < 11$	4·508	6
$> 11 < 13$	3·164	4
$> 13 < 15$	2·016	2
$> 15 < 17$	1·176	—
$> 17 < 19$	·672	—
$> 19 < 21$	·336	1
> 21	·362	2
	28·000	

Testing the goodness of fit we find

$$\sqrt{2\chi^2} = 23{\cdot}907$$
$$\sqrt{2n' - 3} = 22{\cdot}428$$
$$\text{Difference} = 1{\cdot}479$$

showing the fit to be good, and since the correlation between mean infestation and χ^2 is insignificant, we are justified in regarding our samples as random samples from a binomial distribution.

For the whole field $\chi^2 = 561{\cdot}84$ and as $n' = 28$, it is obvious that there are significant differences between plots.

Correlating the infestation means of the plots in 1922 with those in 1923, we find $r = + {\cdot}635$, showing about 64 per cent. of the causes of variation in degree of infestation to be common to both years. All things considered, this is probably as good agreement as one could reasonably expect in experiments of this nature, and may fairly be held as confirming our conclusion as to the differential effect of manures on infestation.

Among the additional counts made in 1923 were those of shoot density, or number of shoots per given area, and number of infested shoots for the same area, in order to test the hypothesis that if the attack is absolutely at random over the whole field, those plots with the greatest shoot density would tend to have a diminished infestation.

The coefficient of correlation showing the relationship between shoot density and number of infested shoots per square yard is $r = - {\cdot}39$. Having regard to the small number of cases (28) this is barely significant; it indicates that increased shoot density does in fact tend to be associated with reduced infestation, but not sufficiently so to make it an important cause of variation in the latter.

The results of the fourfold manurial comparisons are set out in Table X, for comparison with Table VI.

Table X.

Values of χ for fourfold Manurial Comparisons. Hoos Field, 1923.

Superphosphate *v.* unmanured	+6·371
„ with Rape Cake	+·934
„ „ Ammonium Salts (+Potash)	+5·000
„ „ Sodium Nitrate	+8·330
„ „ Alkali Salts	+1·670
„ „ „ „ +Ammonium Salts	+7·272
„ „ „ „ +Sodium Nitrate	+7·800
„ „ Sodium Nitrate+Silicate	+6·740
„ „ „ „ + „ +Alkali Salts	+7·070
„ „ Rape Cake+Alkali Salts	+1·089
Farmyard Manure *v.* unmanured	+7·327
Potash with Superphosphate	−3·809
„ „ „ +Ammonium Salts	−1·212
Sodium Nitrate *v.* unmanured	−2·125
„ „ with Superphosphate	+·407
„ „ „ Alkali Salts	−3·478
„ „ „ Minerals	+2·908
Ammonium Salts *v.* unmanured	−1·066
„ „ with Superphosphate	−2·094
„ „ „ Alkali Salts	−2·911
„ „ „ Minerals	+2·908
„ „ „ Superphosphate+Potash	+·535
Rape Cake *v.* unmanured	+5·172
„ „ with Superphosphate	−·296
„ „ „ Alkali Salts	+2·096
„ „ „ Minerals	+1·515
Minerals *v.* unmanured	+4·280
„ with Ammonium Salts	+7·450
„ „ Sodium Nitrate	+8·692
„ „ Rape Cake	+·647
Alkali Salts *v.* unmanured	+3·513
„ „ with Sodium Nitrate	+·178
„ „ „ Ammonium Salts	+·193
„ „ „ Rape Cake	−·443
Sodium Silicate with Sodium Nitrate	+·644
„ „ „ „ „ +Alkali Salts	+·190
„ „ „ „ „ +Superphosphate	+·306
„ „ „ „ „ +Minerals	−·628

It will be seen that on the whole there is fairly good agreement between the two years' results, the most striking change being that Potash does not appear to have exercised any beneficial effect in 1923. Minerals, Superphosphate, and Farmyard Manure are again markedly beneficial, and it seems highly probable that a more detailed examination of the 1923 results, which lack of time forbids my carrying out, would yield efficiency figures for the various manures (with the exception of Potash) not widely different from those found in 1922. At any rate there is no doubt about the main result, that in any attempt to reduce gout fly infestation on such land as at Rothamsted by means of the application of manures, the best results will be obtained by Farmyard Manure, complete Minerals or Superphosphate. For a full discussion as to the way in which these manures act in bringing about a reduction in infestation, the reader is referred to Mr Frew's paper (4).

In conclusion, I have to acknowledge my indebtedness to Mr R. A. Fisher, in whose laboratory this work was done, and who contributed invaluable help and advice throughout.

REFERENCES.

(1) FISHER, R. A., THORNTON, H. G. and MACKENZIE, W. A. (1922). The accuracy of the plating method of estimating the density of bacterial populations. *Ann. App. Biol.* IX, Nov.

(2) FISHER, R. A. (1922). On the interpretation of χ^2 from Contingency Tables and the calculation of *P. J.R.S.S.* LXXXV, Jan.

(3) —— (1922). The systematic location of genes by means of crossover observations. *The American Naturalist,* LVI, Sept.-Oct.

(4) FREW, J. G. H. (1924). On *Chlorops taeniopus* Meig. (The Gout Fly of Barley). *Ann. App. Biol.* Jan.

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APPENDIX I.

Table showing manurial treatment, number of shoots attacked per hundred examined, mean percentage infestation, and χ^2.

Data for Hoos Field. Barley, 1922.

Plot No.	Manurial treatment	Shoots attacked per 100 examined	Mean infestation %	χ^2
7·1	Unmanured after Dung 1852–1871	18, 26, 18, 20, 16, 14, 17, 27, 13, 13	18·2	14·76
7.2	Farmyard Manure	8, 3, 4, 14, 11, 7, 16, 7, 5, 7	8·2	21·47
6.1	Unmanured	24, 22, 23, 27, 20, 13, 14, 16, 17, 15	19·1	13·25
6.2	20 bushels Ashes	20, 19, 17, 20, 13, 31, 13, 11, 23, 17	18·4	20·13
1.0	No Minerals and no N (*i.e.* unmanured)	13, 25, 17, 17, 18, 21, 21, 21, 9, 17	17·9	12·58
2.0	Superphosphate only. No. N	15, 11, 12, 16, 15, 12, 17, 7, 16, 13	13·4	7·10
3.0	Alkali Salts only. No. N	19, 16, 11, 11, 20, 20, 15, 20, 16, 9	15·7	11·79
4.0	Complete Minerals. No. N	15, 9, 6, 12, 11, 19, 12, 9, 6, 8	10·7	15·50
5.0	Superphosphate + Potassium Sulphate	10, 14, 8, 6, 5, 12, 7, 9, 6, 10	8·7	9·33
1A	Ammonium Salts alone	20, 16, 24, 19, 20, 19, 20, 28, 11, 15	19·2	12·34
2A	Superphosphate + Ammonium Salts	13, 9, 12, 15, 18, 12, 19, 11, 10, 14	13·3	8·34
3A	Alkali Salts + Ammonium Salts	16, 14, 20, 20, 19, 22, 23, 14, 11, 23	18·2	10·72
4A	Complete Minerals + Ammonium Salts	4, 4, 6, 12, 2, 10, 5, 5, 7, 0	5·5	21·64
5A	Potassium Sulphate + Superphosphate + Ammonium Salts	4, 6, 2, 7, 8, 3, 4, 7, 4, 3	4·8	8·22
1AA	Sodium Nitrate alone	21, 19, 15, 13, 31, 22, 19, 18, 22, 15	19·5	14·81
2AA	Superphosphate + Sodium Nitrate	16, 7, 4, 13, 7, 13, 12, 14, 11, 11	10·8	12·83
3AA	Alkali Salts + Sodium Nitrate	12, 11, 14, 8, 19, 15, 14, 11, 9, 9	12·2	9·49
4AA	Complete Minerals + Sodium Nitrate	8, 10, 11, 12, 14, 6, 11, 7, 7, 3	8·9	11·95
1AAS	As 1AA + Sodium Silicate	19, 14, 21, 15, 14, 23, 13, 15, 16, 16	16·6	7·34
2AAS	As 2AA + Sodium Silicate	13, 8, 10, 11, 11, 7, 7, 10, 15, 14	10·6	7·43
3AAS	As 3AA + Sodium Silicate	8, 9, 18, 9, 14, 11, 9, 16, 9, 8	11·1	11·84
4AAS	As 4AA + Sodium Silicate	12, 10, 8, 1, 4, 5, 4, 7, 10, 13	7·4	19·90
1C	Rape Cake alone	20, 22, 15, 25, 10, 25, 21, 15, 21, 16	19·0	13·78
2C	Superphosphate + Rape Cake	10, 11, 10, 9, 8, 7, 10, 16, 6, 9	9·8	6·74
3C	Alkali Salts + Rape Cake	10, 9, 6, 12, 13, 13, 11, 8, 10, 3	9·5	10·53
4C	Complete Minerals + Rape Cake	8, 3, 2, 3, 4, 4, 6, 8, 11, 2	5·1	17·13
1N	Sodium Nitrate alone	6, 9, 10, 13, 10, 12, 14, 23, 16, 17	13·0	18·56
2N	Sodium Nitrate alone	4, 9, 10, 9, 9, 13, 15, 13, 8, 9	9·9	9·74

2N had double the quantity of Sodium Nitrate up to 1857; since then the 1N and 2N plots have had equal quantities.

Data for Woburn. Barley (Stackyard Field).

Plot No.	Manurial treatment	Shoots attacked per 100 examined	Mean infestation %	χ^2
10A	Superphosphate 3 cwts. + Sodium Nitrate (=25 lbs. Ammonia)	21, 26, 19, 18, 10, 14, 22, 20, 15, 17	18·2	12·33
10B	Rape Cake (=25 lbs. Ammonia)	15, 22, 20, 17, 14, 12, 13, 15, 17, 20	16·5	7·15
11A	Sulphate of Potash 1 cwt. + Sodium Nitrate (=25 lbs. Ammonia)	9, 11, 20, 18, 8, 15, 17, 9, 11, 5	12·3	20·22
11B	Farmyard Manure (=100 lbs. Ammonia)	16, 11, 11, 12, 13, 19, 14, 14, 14, 18	14·2	5·55
9B	Mineral Manures and in alternate years Sodium Nitrate (=50 lbs. Ammonia) (applied 1922)	11, 14, 14, 14, 12, 13, 15, 13, 18, 21	14·5	6·33
6	Mineral Manures and Sodium Nitrate (=25 lbs. Ammonia)	19, 14, 18, 17, 17, 16, 10, 13, 21, 19	16·4	7·03
3B	(End limed in 1921) Sodium Nitrate (=25 lbs. Ammonia)	19, 16, 13, 14, 8, 10, 11, 15, 14, 15	13·5	7·75
„	(End not limed in 1921) „ „ („ „)	15, 11, 14, 16, 20, 14, 16, 12, 10, 15	14·3	6·04
3A	(End limed in 1921) „ „ (=50 lbs. Ammonia)	16, 18, 11, 18, 21, 10, 20, 18, 17, 21	17·0	9·22
„	(End not limed in 1921) „ „ („ „)	12, 21, 13, 14, 14, 17, 22, 16, 18, 11	15·8	9·29
5A	Mineral Manures + Sulphate of Ammonia (=25 lbs. Ammonia)	17, 17, 13, 12, 16, 7, 7, 14, 18, 12	13·3	12·15
5AA	As 5A but with 1 ton of Lime March 1905; repeated April 1916	14, 17, 28, 17, 17, 16, 12, 24, 15, 18	17·8	13·92
8B	Mineral Manures and in alternate years Sulphate of Ammonia (=50 lbs. Ammonia) (applied 1922)	5, 6, 7, 5, 8, 13, 3, 5, 6, 5	6·3	11·20
8BB	As 8B but with 2 tons of Lime December 1897; repeated 1912	18, 18, 17, 17, 18, 16, 17, 18, 15, 15	16·9	·91
2A	Sulphate of Ammonia (=25 lbs. Ammonia)	9, 4, 12, 13, 8, 11, 3, 7, 7, 8	8·2	12·43
2AA	As 2A but with 5 cwts. Lime March 1905; repeated 1909, 1910 and 1912	9, 20, 15, 14, 19, 17, 18, 22, 18, 11	16·3	10·86
4A	Mineral Manures alone	12, 20, 14, 16, 17, 19, 20, 17, 19, 11	16·5	6·86
4B	As 4A but with 1 ton Lime March 1915	25, 21, 23, 11, 12, 15, 19, 22, 25, 12	18·5	18·34
7	No Manure	24, 23, 23, 18, 21, 15, 24, 30, 24, 17	21·9	9·87
1	No Manure	17, 22, 20, 27, 19, 22, 20, 23, 16, 17	20·3	6·19

Malting Barley Experimental Plots.

Plot No.	Manurial treatment	Shoots attacked per 100 examined	Mean infestation %	χ^2
1.	*Long Hoos Field, Rothamsted.*			
1	3 cwt. Superphosphate + 1½ cwts. Sulphate of Potash + 1 cwt. Sulphate of Ammonia	14, 18, 14, 9, 13, 12, 10, 11, 15, 19	13·5	8·09
2	1½ cwts. Sulphate of Potash + 1 cwt. Sulphate of Ammonia	17, 16, 16, 10, 16, 14, 19, 16, 12, 15	15·1	4·51
3	3 cwts. Superphosphate + 1 cwt. Sulphate of Ammonia	23, 26, 15, 15, 23, 12, 13, 14, 15, 23	17·9	16·53
4	„ „ + 1½ cwts. Sulphate of Potash	12, 13, 15, 18, 13, 13, 18, 14, 23, 9	14·8	11·07
5	No Manure	11, 16, 13, 12, 8, 15, 13, 13, 14, 14	12·9	4·19
6	3 cwts. Superphosphate + 1½ cwts. Muriate of Potash + 1 cwt. Sulphate of Ammonia	12, 21, 6, 18, 17, 19, 12, 10, 9, 5	13·2	24·50
7	„ „ + 1½ cwts. Sulphate of Potash + 93 lbs. Muriate of Ammonia	16, 15, 9, 12, 16, 12, 19, 23, 12, 10	14·4	13·50
2.	*Woburn Malting Barley.*			
1	No Manure	10, 10, 13, 7, 7, 4, 9, 8, 11, 7	8·6	7·43
2	3 cwts. Superphosphate + 1½ cwts. Sulphate of Potash + 1 cwt. Sulphate of Ammonia	10, 15, 8, 6, 8, 14, 13, 10, 10, 9	10·3	8·02
3	„ „ + 1½ cwts. Sulphate of Potash	10, 13, 9, 4, 2, 15, 9, 11, 8, 9	9·0	16·12
4	„ „ + 1 cwt. Sulphate of Ammonia	13, 8, 13, 15, 11, 9, 12, 13, 10, 9	11·3	4·60
5	1½ cwts. Sulphate of Potash + 1 cwt. Sulphate of Ammonia	13, 15, 12, 10, 11, 7, 10, 11, 4, 10	10·3	9·11
3.	*Dunmow Malting Barley (Mr Hasler's Farm).*			
1	No Manure	4, 7, 3, 7, 7, 2, 6, 2, 4, 7	4·9	8·78
2	3 cwts. Superphosphate + 1½ cwts. Sulphate of Potash + 1 cwt. Sulphate of Ammonia	7, 2, 5, 4, 6, 5, 1, 2, 7, 7	4·6	10·58
3	„ „ + 1½ cwts. Sulphate of Potash	3, 5, 4, 2, 5, 7, 2, 1, 10, 2	4·1	17·52
4	„ „ +1 cwt. Sulphate of Ammonia	2, 4, 3, 1, 4, 0, 4, 2, 6, 3	2·9	9·56
5	1½ cwts. Sulphate of Potash + 1 cwt. Sulphate of Ammonia	1, 1, 3, 5, 7, 8, 3, 8, 3, 5	4·4	14·94
4.	*Wellingore Malting Barley (Mr Nevil's Farm).*			
A	1 cwt. Sulphate of Ammonia + 1½ cwts. Sulphate of Potash	1, 0, 1, 1, 1, 0, 1, 0, 0, 1	·6	4·02
B	No Manure	1, 3, 2, 5, 3, 3, 0, 4, 2, 3	2·6	7·27
C	3 cwts. Superphosphate + 1½ cwts. Sulphate of Potash + 1 cwt. Sulphate of Ammonia	4, 7, 4, 7, 6, 6, 6, 2, 2, 2	4·6	8·75
D	„ „ + 1½ cwts. Sulphate of Potash	0, 3, 2, 0, 2, 1, 4, 4, 6, 5	2·7	14·50
E	„ „ + 1 cwt. Sulphate of Ammonia	3, 3, 4, 3, 0, 3, 2, 1, 2, 8	2·9	14·52

APPENDIX II.

Data for Hoos Field. Barley, 1923.

(Manurial treatment as in Appendix I.)

Plot No.	Shoots attacked per 100 examined	Mean infestation %	χ^2	Infested shoots per sq. yd.	No. of shoots per 2 sq. yds. at time of infestation counts
7.1	14, 24, 21, 11, 11, 17, 23, 13, 16, 18	16·8	14·28	47·1	561
7.2	1, 4, 3, 5, 9, 4, 4, 3, 5, 5	4·3	9·26	16·4	763
6.1	11, 19, 17, 12, 14, 12, 10, 14, 15, 17	14·1	6·35	33·7	478
6.2	14, 11, 14, 12, 15, 20, 10, 14, 14, 16	14·0	5·81	37·9	541
1.0	11, 11, 15, 5, 12, 11, 12, 10, 15, 13	11·5	7·12	28·0	487
2.0	7, 5, 4, 6, 3, 6, 4, 6, 6, 6	5·3	2·81	15·7	594
3.0	8, 10, 6, 8, 12, 8, 12, 7, 9, 17	9·7	10·74	24·6	508
4.0	6, 5, 8, 5, 6, 10, 5, 10, 7, 14	7·6	11·16	19·6	516
5.0	8, 18, 10, 9, 6, 10, 12, 8, 10, 7	9·8	11·49	24·1	493
1A	16, 20, 14, 10, 16, 17, 14, 13, 11, 11	14·2	7·19	36·6	516
2A	5, 8, 4, 4, 9, 14, 6, 8, 7, 11	7·6	12·87	24·6	659
3A	7, 18, 16, 21, 13, 15, 13, 13, 12, 11	13·9	11·27	41·7	600
4A	5, 6, 11, 3, 4, 4, 2, 2, 5, 3	4·5	14·53	14·1	628
5A	8, 3, 10, 16, 13, 5, 14, 6, 6, 10	9·1	19·70	22·1	486
1AA	11, 14, 15, 22, 18, 12, 18, 16, 16, 23	16·5	9·91	49·3	597
2AA	4, 3, 8, 4, 5, 7, 6, 4, 2, 6	4·9	6·63	17·8	727
3AA	11, 13, 10, 11, 18, 21, 15, 15, 18, 16	14·8	9·17	44·7	604
4AA	5, 4, 2, 2, 13, 3, 2, 6, 1, 7	4·5	26·63	13·3	589
1AAS	12, 12, 19, 17, 12, 13, 9, 12, 16, 19	14·1	8·66	42·2	598
2AAS	6, 2, 9, 5, 6, 6, 6, 4, 5, 3	5·2	6·82	17·5	673
3AAS	14, 15, 16, 16, 17, 6, 11, 14, 19, 17	14·5	9·88	36·6	574
4AAS	11, 1, 7, 6, 7, 2, 3, 2, 12, 0	5·1	32·42	18·8	736
1C	7, 8, 6, 4, 7, 6, 4, 8, 8, 8	6·6	3·64	20·1	609
2C	5, 3, 7, 7, 6, 4, 7, 6, 7, 4	5·6	3·86	18·2	652
3C	5, 3, 7, 9, 7, 8, 10, 7, 8, 7	7·1	5·29	21·7	611
4C	3, 4, 9, 6, 2, 8, 8, 7, 4, 8	5·9	9·89	18·6	629
1N	14, 14, 16, 14, 11, 10, 15, 10, 12, 13	12·9	3·46	37·8	586
2N	13, 14, 12, 16, 20, 14, 19, 16, 17, 13	15·4	4·94	40·8	530

THE ACTION OF SOME ORGANIC COMPOUNDS UPON THE CUCUMBER-HOUSE WOODLOUSE

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(With 1 Text-figure.)

IN the search for a good means of controlling the Woodlouse (*Armadillidium speyeri* Jackson) which infests cucumber houses, and which attacks the cucumber plant to a serious extent, the action of a number of aromatic compounds has been tested, with a view to obtaining some substance which would kill these Crustacea in the soil during the winter months.

It is a habit of the woodlice to bury themselves in heaps of soil near glasshouses in the winter, and they are thus brought into the houses in great numbers, during the growing season, in soil used for propagating seed and for making up borders.

To ensure a true standard of comparison, solid substances were chosen throughout. In the experiments an attempt was made to distinguish between contact and vapour action. Owing to the possibility of a vapour condensing on the surface of the animal, these two effects could in no case be sharply differentiated. It was clear that death of the woodlice was not produced in the same way by chemically allied compounds, so that no relation between chemical constitution and action on the animal is to be expected. The results of the experiments, indeed, give us reason to believe that no such correlation exists.

Three sets of conditions were employed in testing for the action of each compound.

In the first, the woodlice came into direct contact with the pure substance; in the second, direct contact was avoided so that the woodlice were exposed to the vapour. In the third, the woodlice were kept in soil with which each substance had been intimately mixed.

A list of the compounds used will be found in the experimental portion of this paper. The most interesting phenomena shown by the experiments were:

(1) The extreme rapidity of death by contact and vapour when

Phenol and *o*-Cresol were used, and the great length of time over which both were operative when mixed with soil, especially the former.

(2) The slow action of the para- as compared with corresponding ortho-compounds used.

(3) The effects of different compounds on different parts of the animal tissues, some affecting the nervous system, others the respiratory system (*e.g.* Hydroquinone), another (Camphene) causing rapid death only by contact.

(4) The greater resistance of some individuals, as compared with others, to the action of any one substance, especially in the case of slow-acting compounds such as Hydroquinone, *p*-Nitrophenol, or Camphor.

(5) The greatest activity of several compounds, *e.g.* Hydroquinone and Camphor, being shown shortly before their disappearance from the soil with which they have been mixed.

(6) The rapid disappearance of Naphthalene when mixed with soil, and the prolonged action of *α*-Naphthylamine under similar conditions.

When subjected to the action of a substance which has a deleterious effect upon them, the woodlice pass through a more or less prolonged period of paralysis, losing control of the flexor and extensor muscles of the body. When paralysis is complete in the latter, the legs and antennae continue to twitch for a considerable time: if removed to normal conditions in this state revival may sometimes take place, but no case has been observed in which a woodlouse recovered after all movement had ceased.

In the case of *α*-Naphthylamine the period of paralysis before death was reduced to a minmium, being often practically unrecognisable. It is not always possible to distinguish between a respiratory and a nervous action, but where death was preceded by a phase in which the gill-plates became brown or blackish in colour, where the abdominal segments were much extended, and the outer division of the tail-appendages were turned upwards, it was concluded that death finally occurred through derangement of the respiratory system. Where the quicker acting substances were used, it appeared that the nervous system became disordered through the nerve-endings in the carapace, and upon the surface of the animal in general. With regard to the slow action of many compounds when mixed with soil, it is evident that a considerable time elapses before the soil-particles become generally invested with the compound in question, and in some cases this might closely precede the time at which the substance begins to disappear.

Further, it is at this period of saturation when the woodlice would be likely to come most often into contact with minute particles of the compound, resulting in their more rapid death. That such contact does occur is clearly evident by the action of both ortho- and para-nitrophenol, where the thoracic legs and gill-plates are stained yellow upon the surface which actually comes into contact with the soil most frequently.

The length of time for which any substance remains operative in affecting woodlice in the soil has been tested by introducing the animals into such soil at definite times after mixing. The results have been standardised from the number of deaths amongst a fixed number of woodlice during a period of 24 hours. The comparative persistency of the most active substances mixed in equivalent proportions in the soil will readily be seen from the curves in the figure.

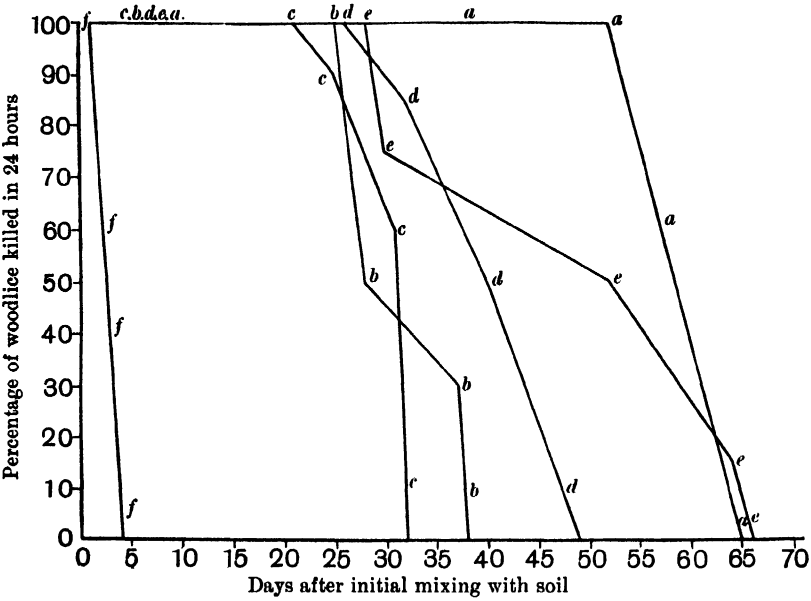

Fig. 1. Curves showing the duration of action on woodlice of substances when mixed with soil: (*a*) phenol, (*b*) *o*-cresol, (*c*) *p*-cresol, (*d*) *o*-nitrophenol, (*e*) α-naphthylamine, (*f*) naphthalene.

Each of the compounds illustrated killed, in the experiments, 100 per cent. of the woodlice introduced in a 24 hours' period of time:

Phenol	for 52 days	*o*-Nitrophenol	for 26 days
α-Naphthylamine	,, 28 ,,	*o*-Cresol	,, 25 ,,
Naphthalene	,, 3 ,,	*p*-Cresol	,, 20 ,,

All the compounds are injurious to plant growth, and must therefore be allowed to disappear from the soil before sowing or planting. In the case of Phenol, it has been found that tomato plants will germinate practically normally if the seed is sown 20 days after mixing at the rate of 1 part Phenol in 750 parts of soil. This amount is sufficient to kill 100 per cent. woodlice for at least six days after mixing.

EXPERIMENTAL.

The compounds were tested in three different ways upon the woodlice, the following actions being observed. (All compounds tested were solid.)

(*a*) Action by direct contact.
(*b*) Action by vapour.
(*c*) Action of the substance when intimately mixed with the soil.

In testing (*a*), a small amount of each substance was placed at the bottom of a wide glass tube, and five woodlice dropped in, the tube being left open at the mouth. The experiments were made at room temperature.

In (*b*), similar methods were employed, but a thin layer of glass wool about half-an-inch above each substance prevented the animals from touching the latter. Further, the tubes were plugged with moist cotton-wool and placed in a hot-house.

In the case of (*c*), 250 grammes of fresh soil were thoroughly mixed with one-hundredth part of the molecular weight of each substance in grammes, the mixture being placed in a glass jar 8 inches high and 3 inches in diameter. Ten woodlice were placed on the surface of the soil, more being added at required intervals.

Controls were set up in each case, as these woodlice are liable to be adversely influenced by lack of food over long periods of time. Into some of the soil jars, 20 c.c. of water were added from time to time to prevent any serious desiccation.

In the controls whether water was added to the soil at the beginning of the experiment or not, the woodlice were all living 29 days after being placed in the jars, all being dead on the 36th day, but if separated from the soil, by a piece of wire gauze, they began to die after only four days, and none survived six days. Again, if water was added to the soil after being 29 days in a jar, and fresh woodlice were introduced, they seldom survived more than four days. This all points to exhaustion of food in the soil, and not to lack of moisture, as being the cause of death.

It is readily admitted that in some cases, death may have resulted from starvation through contamination of the food-supply by a compound used, though in only one case (Phenanthrene) could death have been attributed to actual poisoning through the intestine. This would account for the comparatively large number of deaths in soil from which a substance has presumably almost disappeared.

Action by Contact.

The total duration of these experiments was 17 hours. At the end of this period, one out of the five woodlice used was dead in the control. When any of the woodlice in the tubes were still living at the end of the experiment, the substance employed was deemed to have no action.

In the following tables the maximum and minimum times in which death and paralysis are brought about by the various compounds are given:

Contact Action. Temperature 64–72° F.

Death in minutes	Paralysis in minutes	Substances
1– 2	1	Phenol, *o*-Cresol, *p*-Cresol
6– 36	2– 6	*p*-Nitrophenol, Camphene
33– 62	31– 41	*p*-Nitrophenol
60– 75	16– 38	Thymol, Camphor
75– 90	20– 70	Pyrogallol, Resorcinol
90–150	24– 60	Pyrocatechol, *α*-Naphthol, Naphthalene
150 upwards	40–100	Hydroquinone, *β*-Naphthol, Menthol

In the case of Phenanthrene, there was a slight contact action, two woodlice out of five still showing signs of movement at the end of 17 hours. Pyrocatechol, *α*-Naphthol and Hydroquinone tended to darken the gill-plates. The Nitrophenol stained the appendages and carapace yellow. Anthracene, Anthraquinone, Alizarin, Phloroglucinol and Borneol were without action.

Action by Vapour.

The duration of the experiments was limited to 21 hours. In the controls, which consisted of one tube plugged with dry and another with wet cotton wool, the woodlice were all alive at the end of this time.

Vapour Action.

Death in minutes	Paralysis in minutes	Temperature ° F.	Substances
5 – 17	1½–12	70– 96	*o*-Cresol
24 – 34	3½–19	70– 98	Phenol
27 – 50	7 –30	70–100	*p*-Cresol
50 –100	23 –75	70– 90	Camphor, Camphene, *o*-Nitrophenol, Thymol
150 upwards,			Menthol, *α*-Naphthylamine, Naphthalene, Anthracene, Anthraquinone
Also tested			
8 – 50	2 –14	69– 90	*o*-Dichlorbenzene
12 – 90	6 –42	69– 90	*p*-Dichlorbenzene
9¼– 17	6½–13½	86	Pyridine

The following were without action: *p*-Nitrophenol, Hydroquinone, Resorcinol, Pyrocatechol, Pyrogallol, Phloroglucinol, *α*-Naphthol, *β*-Naphthol, Alizarin, Borneol. *o*-Nitrophenol stained the woodlice yellow. The appendages were much blackened by Anthraquinone. The inactivity of Hydroquinone shows that this substance acts by contact upon the gill-plates of the animals, causing death by affecting the external respiratory system.

Action in the Soil.

The controls have already been referred to, and curves given of the quicker acting compounds. All the compounds were carefully mixed with 250 grammes soil in the following proportions, and the experiment carried out at room temperature, except when otherwise stated:

Phenol 1 part in 265 parts soil.
o-Cresol, *p*-Cresol, 1 part in 231 parts soil.
o-Nitrophenol, *p*-Nitrophenol, 1 part in 108 parts soil.
Resorcinol, Pyrocatechol, Hydroquinone, 1 part in 227 soil.
Pyrogallol 1 part in 198 soil.
Phloroglucinol 1 part in 154 soil.
Naphthalene 1 part in 195 soil.
α- and *β*-Naphthol 1 part in 173 soil.
α-Naphthylamine 1 part in 174 soil.
Anthracene, Phenanthrene, 1 part in 140 soil.
Alizarin 1 part in 104 soil.
Anthraquinone 1 part in 120 soil.
Camphene 1 ,, 183 ,,
Camphor 1 ,, 164 ,,
Menthol 1 ,, 160 ,,
Borneol 1 ,, 162 ,,
Thymol 1 ,, 166 ,,

Resorcinol, Pyrocatechol, Pyrogallol, Phloroglucinol, *β*-Naphthol, Anthracene, Alizarin, Anthraquinone, had no action; Borneol killed 30 per cent. during the first five days, Camphene 40 per cent. during the first three days, the action ceasing in each case at the period mentioned.

Phenol. 100 per cent. woodlice killed within 24 hours up to the 52nd day after mixing. (On the 44th day after mixing paralysis set in 1¼ hours after the woodlice were put in the jar.)

Concentration 1 part in 500 soil (25 c.c. of a 2 per cent. solution in water). 100 per cent. woodlice killed within 24 hours up to the ninth day after mixing. Action ceases on the 14th day after mixing.

Concentration 1 part in 750 soil (20 c.c. of a 1·65 per cent. solution in water). 100 per cent. woodlice killed within 24 hours up to the sixth day after mixing. Action ceases on the ninth day after mixing.

Concentration 1 part in 1000 (25 c.c. of a 1 per cent. solution in water). 80 per cent. woodlice killed in the first 24 hours after mixing, then

100 per cent. killed in the second 24 hours after mixing. Action ceases on the fourth day after mixing.

A concentration of 1 part Phenol in 2000 parts soil had no action.

o-*Cresol.* 100 per cent. woodlice killed within 24 hours up to the 25th day after mixing. Action ceases on the 38th day after mixing.

p-*Cresol.* 100 per cent. woodlice killed within 24 hours up to the 21st day after mixing. Action ceases on the 32nd day after mixing.

o-*Nitrophenol.* 100 per cent. woodlice killed within 24 hours up to the 26th day after mixing. Action ceases on the 49th day after mixing. The appendages and gill-plates are stained yellow.

p-*Nitrophenol.* Slow action. 100 per cent. killed in 10 days up to the 20th day after mixing. Maximum activity from 20–23 days after mixing. Action ceases 55 days after mixing. The appendages and gill-plates are stained yellow.

Hydroquinone. 100 per cent. killed in three days up to the fifth day after mixing. Maximum activity from sixth to eighth day after mixing. Action ceases on the tenth day after mixing. In solution in water at equivalent strength the maximum activity takes place from fifth to seventh day and action ceases on the eighth day after mixing. At hot-house temperature, the maximum activity was shown from fourth to sixth day, action ceasing on the seventh day after mixing.

A characteristic attitude is taken up by the woodlice when placed in the jars; they remain on the surface of the soil, and after 24 hours they become inactive, the segments of the thorax and abdomen are extended, but compressed from side to side. The thoracic appendages move weakly, and the outer division of the tail appendages are held upwards and outwards. The gill-plates of the abdomen become dusky, and turn brown shortly before death. It has been observed that the dark colour extends into the tracheal tubes connected with the gill-plates. It is concluded that Hydroquinone enters the system through actual contact with the abdominal appendages and passes from them into the respiratory system. Deaths in the jars from the eighth to seventeenth day after mixing indicate lack of nutriment.

Naphthalene. 100 per cent. woodlice are killed in the first 24 hours after mixing. The action then becomes slightly slower and ceases on the fourth day. It is probable that the nerve-endings on the surface of the animal are affected: paralysis sets in early and is of considerable duration before movement ceases.

a-Naphthol. Action starts within the first 24 hours, and is at its maximum till the fifth day after mixing. Whereas most are killed during the first two days, some will survive for eight days. From the second to fourth day, the action is more definite, but later the woodlice die irregularly, some surviving for at least 11 days after being placed in the jars. The action appears to cease on the ninth day after mixing. Similar results were obtained at hot-house temperature.

a-Naphthylamine. 100 per cent. woodlice are killed within 24 hours up to the 28th day after mixing. The rate of action is then about halved till the 64th day, and after the 66th day from mixing action ceases. The period of paralysis before death is extremely short, and it is possible that death is produced by vapours entering the respiratory system.

Phenanthrene. Experiments at hot-house temperature. The action is slow and very irregular. From the large number of deaths from the ninth day after mixing onwards, it is probable that the woodlice eat this compound when the food supply becomes scanty. No definite paralysis preceded death, the movement simply becoming feeble and the segments of the animals contracted.

Camphor. Experiment at hot-house temperature. The action is weak, 90 per cent. being killed from the first to fifth day after mixing, with a maximum activity (100 per cent. in three days) from the third to fifth day; after the sixth day, there appeared to be no action. Some woodlice were entirely unaffected from the start of the experiment, and seemed more resistant than others.

Menthol. Experiment at hot-house temperature. The action is at first slow, and gradually increases to the ninth day after mixing. It was not determined how long the activity lasted.

Thymol. Experiment at hot-house temperature. The action is at first slow, but after the third day from mixing, 100 per cent. woodlice are killed within 48 hours until the ninth day. Some woodlice are much more resistant than others when placed in the soil at the start of the experiment, but later the action becomes definite.

SUMMARY.

With a view to the winter control of the cucumber woodlouse the action of a number of compounds has been tested. Observations are described on the effects due to contact, vapour and mixing with the soil.

The organism of the animal is not affected in a similar manner by different compounds. *p*-Cresol and *p*-Nitrophenol are less active than the corresponding ortho-compounds and both nitro-phenols are less active than phenol.

Certain compounds show a maximum activity shortly before their disappearance from the soil. Phenol and the cresols were the most active compounds tested. Naphthalene ceases to act in the soil on the fourth day after mixing.

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A ROOT DISEASE OF THE TOMATO CAUSED BY *COLLETOTRICHUM TABIFICUM* (HALLIER *PRO PARTE*) PETHYBRIDGE

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AND J. SHEARN.

(With Plates V and VI.)

During an investigation of tomato root-rots in 1919, cases of a distinctive type were met with in which very numerous, minute, black sclerotia were found embedded deeply in the woody tissues. The causal organism was isolated, its pathogenicity proved and cultures were set aside for further study when circumstances permitted. In 1920 a culture of the same fungus, which he had isolated from a diseased tomato root, was sent to us by Dr H. Wormald. In 1921 the disease assumed increased importance and in the Lea Valley it was continually found during the summer. When roots were examined at the end of the season, many hundreds were discovered in an advanced state of decay, and containing the sclerotia. Further information concerning the disease was obtained in 1922, when a systematic inspection of commercial nurseries indicated that it was of considerable economic importance in nurseries where tomatoes had been grown continuously for several years.

During the season of 1923 the disease appeared unusually early and cases of death from this cause were observed in May and June. At this time it was apparent that the constitution of the tomato crop had been appreciably weakened by the unfavourable weather conditions which had prevailed. This became accentuated later by the absence of sufficient sunlight to ripen the fruit quickly and permit of its being gathered, and the heavy trusses of green fruit thus remaining caused a severe drain on the plants.

SYMPTOMS OF THE DISEASE.

The symptoms of the disease do not differ in any material respect from those usually exhibited by tomato plants, the roots of which are being slowly destroyed by other parasitic fungi. Such plants cease to develop in a normal manner; the lowest leaves turn yellow and die prematurely, while any new growth is weak and pale in colour. In advanced stages, the stem becomes yellow and hollow, while a dark brown discoloration sometimes appears on the outside of the stem near the ground and spreads rapidly up the plant. Usually the discoloured streak is about a quarter of an inch in width and occurs on one side of the stem only. On pulling up a diseased plant, the roots offer no resistance, as they are dry and shrivelled; and the decayed base of the stem separates readily from the soil. If the roots are carefully dug out, they are seen to be completely decayed and brown. The tissues external to the wood are destroyed and shrivelled, and fit quite loosely over the central woody cylinder. The distinctive feature is the presence of numerous minute, black sclerotia both external to and embedded in the wood (Pl. V, fig. 1). These are most commonly confined to the roots, but in bad cases the base of the stem below ground and even its lower aerial part may contain sclerotia. The infected wood varies from "Light Brown[1]" in the root to "Wood Brown" in the stem, and the wood of the stem may be discoloured up to a distance of two and a half feet from soil level. The colour of the darkest part of the discoloured stem wood is "Saccardo's Amber" in cross section and "Wood Brown" in longitudinal section, while, not infrequently, a beautiful amethystine colour pervades the wood. Sclerotia are most numerous in the roots but they have been found in the stem wood as far as 28 inches above the soil. When situated on the outside of the wood or in pith cavities, the sclerotia are liberally provided with stiff black hairs or setae, which are absent when these bodies are formed within the wood elements.

MORBID ANATOMY.

Infection takes place through the roots, and a wound does not appear to be necessary. The finer roots are first attacked, and the fungal hyphae spread rapidly through the tissues up to the stem base and finally may pass into the aerial portions of the stem, where they have been found up to 30 inches above ground level.

Investigation has shown that the root cortex is first attacked, the rapid destruction of the cells leaving only a hard dry envelope lying loosely round the central woody core. The invading hyphae readily enter the woody tissues, and, although not confined to any particular type of element they are generally most abundant in the larger vessels. Except in the immediate vicinity of the sclerotia, the hyphae are hyaline, but their component cells are frequently swollen and irregular in shape indicating a tendency to produce sclerotia. These latter bodies are produced mainly when the hyphae reach the larger vessels or the exterior and interior of the woody cylinder where the cortex or the pith respectively has been destroyed. A sclerotium within a vessel gradually increases in size until the lumen is completely filled, and in such situations setae are rarely produced. The hyphae on the outside of the sclerotium do not in this case form a dense dark layer but continue to develop, and, by passing through the pits in the vessel-walls, grow into the surrounding elements. Thus a large sclerotium may in time extend over a considerable number of wood elements. Whilst the tendency of development is to follow the line of least resistance by spreading up and down the vessels, so that longitudinal sections show the sclerotia as being long and narrow, considerable spreading also takes place transversely. As a general rule the walls of the xylem elements surrounded by sclerotial masses show no sign of undergoing change by reason of fungal secretion, but occasionally the walls are seen to be blackened and are apparently brittle.

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[1] Ridgway, *Color Standards and Nomenclature.*

Sclerotia formed on the outside of the wood or within the pith cavity are more regular in shape than those produced internally, and are usually provided with setae.

Comparative observations have shown that the wood of tomato plants infected by this fungus contains a considerably greater amount of mycelium than that of plants attacked by either *Fusarium lycopersici* or *Verticillium albo-atrum.*

INOCULATION EXPERIMENTS.

No difficulty was experienced in isolating the causal organism, for it readily grows out from small pieces of diseased wood removed aseptically and placed in the centre of poured potato-agar plates. Typical sclerotia are produced on this medium in five or six days. The organism grows well on artificial media and on sterilised vegetable tissue (Pl. V, fig. 2). Its pathogenicity was readily proved by growing healthy plants in sterilised soil copiously contaminated with sclerotia obtained from a pure culture. The lower leaves of such plants became yellow and drooped within six weeks of planting, while those of suitable controls remained green and turgid. Within three months of planting, ten plants out of twelve were stunted in comparison with the healthy controls. At this time the roots of all twelve plants were in an advanced state of decay, showing typical sclerotia, and the parasite was readily re-isolated. The roots of the control plants were perfectly healthy and showed no signs of sclerotia, while all attempts to isolate a parasitic organism from them failed.

Attempts were made to infect healthy tomato plants (eight weeks old) by inoculating the aerial portions with pieces of mycelium from a pure culture, but with the exception of a browning of the tissues for a distance of about 1 cm. round the inoculation stab, and the consequent production of sclerotia within this restricted area, no positive results were obtained. On the other hand, infection was readily produced by stab inoculations on healthy roots, when the stabs were covered with tin foil and the soil replaced.

Tomato fruits both green and red are quickly rotted by the fungus, if the latter is introduced through a wound. In green fruits a soft brown area is produced round the inoculation point by the second day. This area rapidly spreads, and by seven days after inoculation it may extend half over the fruit. The hyphae ramify through the soft flesh forming an intricate network on which sclerotia are produced freely. The hyphae also break through the skin producing numerous sclerotia on the outside. Natural infection of the fruit has not been observed.

In view of these results it would appear that the disease is contracted in the soil, and that primary infection of the aerial portions rarely, if ever, occurs.

THE CULTURAL CHARACTERS AND IDENTITY OF THE CAUSAL ORGANISM.

In a previous note (1) the fungus causing this disease was described as a new species, *Sclerotium setosum* Bewley and Shearn. Some months after publication a typical *Colletotrichum* fructification was observed on an old culture on cellulose agar, and this led to further investigation.

The characters of the fungus in question recall the published descriptions of Ducomet and others (4, 5, 7) relating to a disease of potatoes and tomatoes due to *Vermicularia varians* Ducomet, and O'Gara's description (8) of *Colletotrichum solanicolum*. They also resemble those of *Colletotrichum tabificum* (Hallier *pro parte*) Pethybridge, described by the latter as a weak parasite on the potato in Ireland; but the consistent absence of any organised condial fructification during three years' cultivation in the laboratory seemed to distinguish it as a separate entity. Pethybridge (9) in describing his investigations concluded that his fungus was identical with that described by Hallier (6) as *Rhizoctonia tabifica*, and noted its similarity to *Vermicularia varians* Ducomet.

The tomato fungus grows readily upon a wide range of media, and for laboratory work, Dox's agar with 1 per cent. saccharose has proved satisfactory. Growth on this medium is rapid and the mycelium develops as a white compact layer over the surface of the medium. Within 14 days of inoculating the medium, the fungal growth assumes a "Shrimp Pink" colour, which remains for 7 to 10 days and then disappears, leaving innumerable minute "Shrimp Pink" masses about 200μ in diameter. These masses slowly increase in size, lose their pink colour and turn black.

The mycelium consists of branched septate hyphae, generally well filled with granular cell contents, which stain deeply with neutral red, and oil drops are plainly visible. Swollen cells, round, oval and pear-shaped are present in the mycelium, and vary in quantity in accordance with the medium. Thus upon inulin agar, casein agar, ethyl butyrate agar and Dox's agar with 1 per cent. saccharose, but few swollen cells are found, while on amygdalin agar, blood fibrin agar, and tyrosin agar they are abundant.

Conidia are produced either on conidiophores or in acervuli. The conidiophores bearing sessile conidia appear in young cultures some 24 hours after seeding with spores or sclerotia, and are also found at the edges of rapidly growing cultures, or at the tops of sloped tube cultures. This is the commonest form of spore production, but occasionally in old cultures both *Gloeosporium* and *Colletotrichum* fructifications may be found, the former direct on the medium, the latter on the surface of the sclerotia. The *Colletotrichum* acervuli appear as watery, dark or amethystine coloured globules surmounting old setae-bearing sclerotia usually at the bottom of a tube slope. Frequently, in hanging drop cultures of germinating spores, appressoria are produced, but perhaps the most characteristic feature of this fungus is the rapid production (usually in 7 to 10 days) of minute black sclerotia on most media. These are similar in appearance to those found on the plant, but whereas here (at least when they are on the exterior and not embedded in the wood) they are liberally provided with dark brown setae, on most media setae are absent. The number of sclerotia produced on each square centimetre of the plate, and the modal size of the sclerotia vary with the kind of medium, as shown in Table I.

The appearance of the *Colletotrichum* fructifications previously mentioned led to further examination of the tomato fungus in parallel with *C. tabificum* and *V. varians*, cultures of which were kindly supplied by Dr G. H. Pethybridge.

Table I.

Medium	Average no. of sclerotia per sq. cm.	Modal size of sclerotia in microns	Percentage of small sclerotia 90 × 100μ in size
Aesculin agar	19	230 × 240	20
Amygdalin agar	159	250 × 300	Centre of Plate 0, edge 80
Arbutin agar	None	None	None
Blood fibrin agar	26	230 × 240	3
Casein agar	35	240 × 250	40
Cellulose agar	31	360 × 450	5
Dox's agar with 1 % saccharose	94	520 × 570	10
Ethyl butyrate agar	55	150 × 170	60
Inulin agar	72	330 × 420	15
Starch agar	39	300 × 400	12

Single spore cultures of the three fungi were grown on seven different media. Plates containing a uniform thickness of medium were prepared in triplicate, and inoculated by placing a sclerotium from a single spore culture in the centre of each plate. After 56 days' incubation at laboratory temperature, six counts of the numbers of sclerotia were made in each plate, and the average of three plates recorded. The results are given in Table II.

Table II.

Showing number of sclerotia per sq. cm. of medium after 56 *days.*

Medium	*Colletotrichum tabificum*	*Sclerotium setosum*	*Vermicularia varians*
Aesculin agar	25	19	26
Amygdalin agar	185	156	124
Arbutin agar	None	None	None
Casein agar	47	35	72
Ethyl butyrate agar	25	34	46
Inulin agar	91	72	85
Starch agar	52	39	48

The amethyst coloration emphasised by Pethybridge (9) appears irregularly in cultures from all three sources, but does not seem to be a constant feature. These results indicate a marked degree of similarity between the three fungi (Pl. VI). They all produce sclerotia and conidia both on conidiophores and in acervuli. Dickson (3) in 1923 described the occurrence of a saltation in cultures of the organism causing "Black Dot" (*Vermicularia varians*) disease of potato tubers in Canada. This is of interest in view of the fact that cultures of the three organisms on Dox's agar with 1 per cent. saccharose have all shown a marked tendency to this phenomenon.

In a recent paper (2) Cavadas, working with *V. varians*, reports the discovery of specialised conidiophores bearing sessile conidia. From his description and figures it is apparent that these are the same bodies as are found in cultures of the tomato fungus. He records further the development of *Colletotrichum* acervuli on media rich in readily assimilable salts, but was unable to find *Vermicularia* structures. This is in complete accord with our own experiences.

Further evidence of identity was sought in inoculation experiments. Sterilised soil was contaminated by the liberal incorporation of steamed tomato-stem cultures of the fungi in sets of twelve, an equal number of non-contaminated controls being left. Six tomatoes and six potatoes were planted in each set of twelve pots. The roots were removed two months later, and obvious infection was found in those from the soil contaminated with all three fungi, while those from the uncontaminated pots were perfectly white and healthy. These results (Table III) coupled with those obtained by the cultivation of the three fungi in various media lead to the conclusion that they are identical.

Table III.

Plant	No. used	Date planted in contaminated soil	Date examined	Fungus employed	No. of plants infected	No. of plants healthy
Tomato	6	2. x. 23	28. xi. 23	Control	—	6
,,	6	,,	,,	*C. tabificum*	5	1
,,	6	,,	,,	*S. setosum*	6	0
,,	6	,,	,,	*V. varians*	5	1
Potato	6	,,	,,	Control	—	6
,,	6	,,	,,	*C. tabificum*	4	2
,,	6	,,	,,	*S. setosum*	5	1
,,	6	,,	,,	*V. varians*	4	2

The question of nomenclature still remains. In view of the fact that no observer has been able to repeat Ducomet's findings of *Vermicularia* fructifications, and that *Colletotrichum* acervuli are constantly obtained, the placing of the fungus in the latter genus seems justified. It is possible that O'Gara's *C. solanicolum* is identical with the fungus now being dealt with, but the absence of inoculation trials leaves this question in doubt. At the same time examination of Hallier's description and figures (6) illustrating *Rhizoctonia tabifica* leaves little doubt that Pethybridge was correct in assuming Hallier's fungus to be identical with that which he isolated from the potato. It is considered therefore that *Colletotrichum tabificum*, *Sclerotium setosum* and *Vermicularia varians* are identical with *Rhizoctonia tabifica* and that the name *Colletotrichum tabificum* (Hallier *pro parte*) Pethybridge should stand.

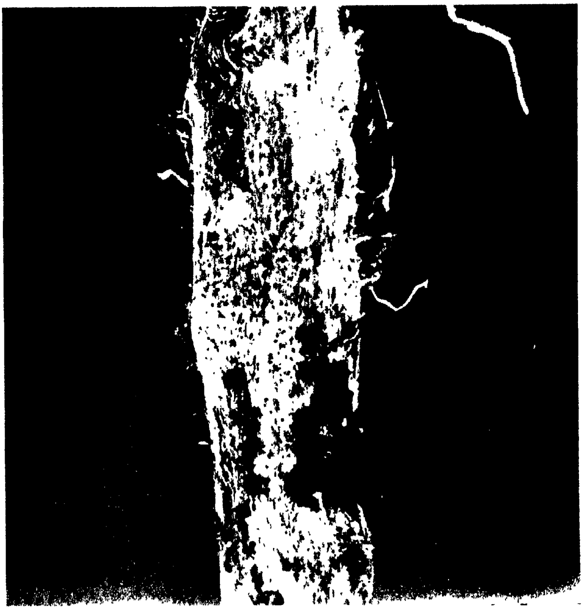


Fig. 1

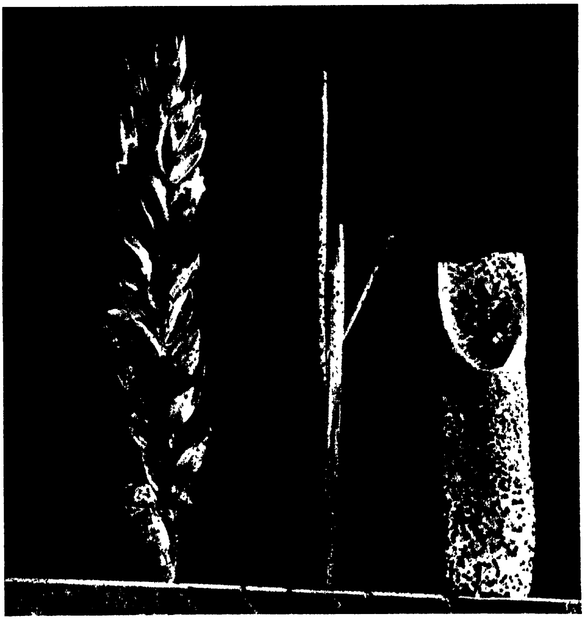
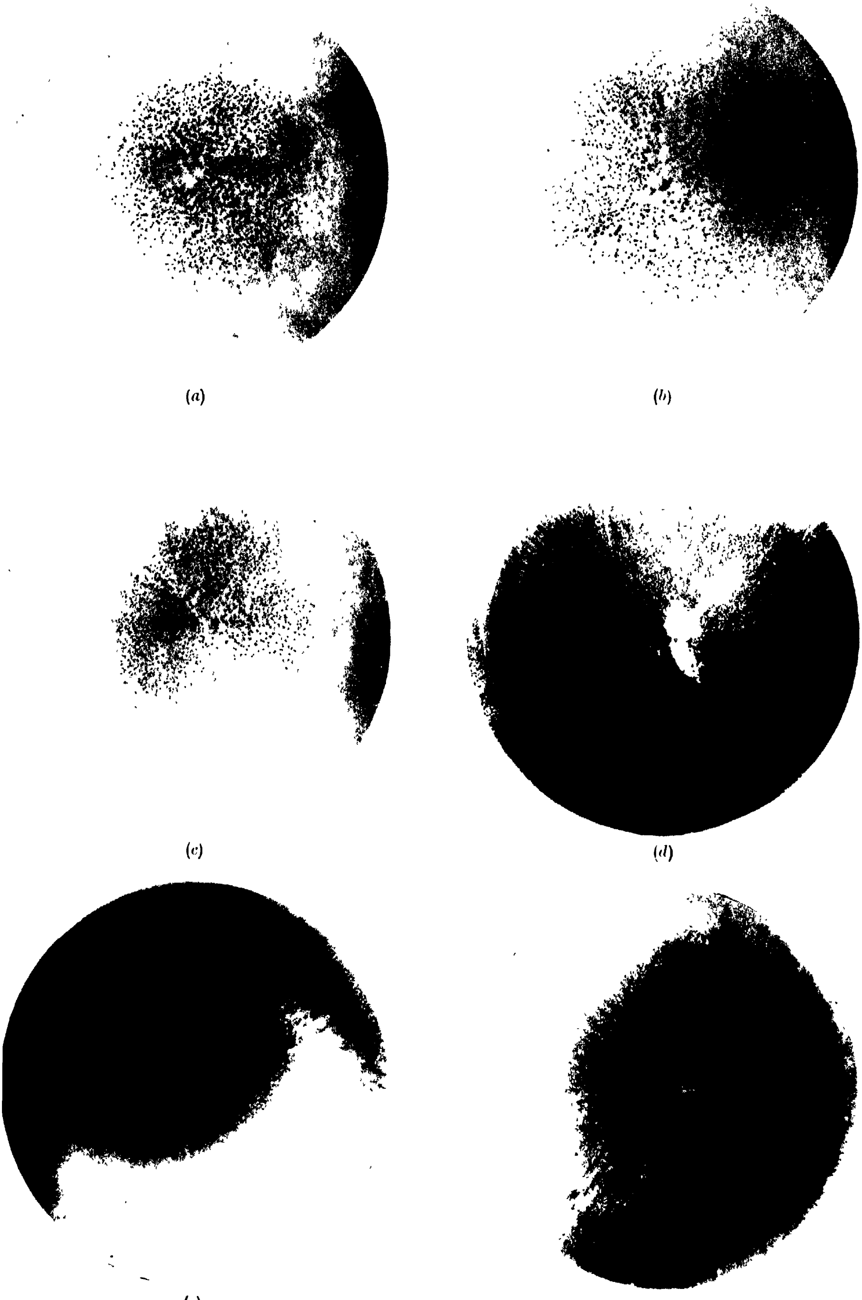


Fig. 2

BEWLEY & SHEARN.—A Root Disease of the Tomato caused by *Colletotrichum tabificum* (Hallier *pro parte*) Pethybridge. (pp. 244—251.)



(*a*) (*b*)

(*c*) (*d*)

(*e*) (*f*)

BEWLEY & SHEARN.—A Root Disease of the Tomato caused by *Colletotrichum tabificum* (Hallier *pro parte*) Pethybridge. (pp. 244—251.)

CONTROL OF THE DISEASE.

At the present stage little can be said about the control of the disease in question, but further investigations are in progress, the results of which will be given later.

SUMMARY.

1. A root disease of the tomato, due to *Colletotrichum tabificum* (Hallier *pro parte*) Pethybridge, has been investigated and described.

2. The pathogen is considered to be identical with *Rhizoctonia tabifica* Hallier, *Vermicularia varians* Ducomet, *Sclerotium setosum* Bewley and Shearn, and possibly *Colletotrichum solanicolum* O'Gara.

REFERENCES.

(1) BEWLEY, W. F. and SHEARN, J. (1922). *Cheshunt Exp. and Res. Stat. Ann. Rep.* VIII, 34.
(2) CAVADAS, D. S. (1923). *Rev. Path. Veget. et D'Ent. Agric.* x, 1, 67.
(3) DICKSON, B. T. (1923). *Trans. Roy. Soc. Canada*, XVII, 123.
(4) DOIDGE, E. M. (1914). *Agr. Journ. Un. South Africa*, VII, 6, 879.
(5) DUCOMET, V. (1908). *Ann. Ecole Nat. Agric. Rennes.* II.
(6) HALLIER, E. (1875). *Zeits. f. Parasitenkunde*, IV, 97.
(7) MCALPINE, D. (1911). *Potato Disease in Australia*, 92.
(8) O'GARA, P. J. (1915). *Mycologia*, VII, 38.
(9) PETHYBRIDGE, G. H. (1919). *Trans. Brit. Mycol. Soc.* VI, 2, 107.

EXPLANATION OF PLATES V AND VI

Plate V.

Fig. 1. Lower portion of diseased tomato stem showing the minute black sclerotia of *Colletotrichum tabificum.*

Fig. 2. Cultures of *C. tabificum* on wheat straw and Dox's agar.

Plate VI.

(*a*) *C. tabificum* on starch agar.
(*b*) *S. setosum* „
(*c*) *V. varians* „
(*d*) *C. tabificum* on Dox's agar.
(*e*) *S. setosum* „
(*f*) *V. varians* „

(*Received March* 5*th*, 1924.)

INVESTIGATION OF THE STRUCTURE OF REVERTED BLACK CURRANTS

BY WINIFRED F. F. RIDLER, M.Sc.

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(With 4 Text-figures and Plate VII.)

LEES (2) described a method for identifying cases of Reversion in Black Currant plants by means of certain morphological characteristics, namely, the number of sub-main veins on the leaf, and the nature of the leaf margin; the number of sub-main veins running from the midrib to points in the margin; and the number of margin points being numerically fewer in reverted leaves than in normal ones.

In examining black currant plants Lees found that there were three main classes: normal healthy plants, "simple reverted" where there is apparently no mite infection, but where mite infection has probably been at work, and the plant is still suffering from the effect of some previous infection; and "mite infection (reverted)" where mite is still present. Corresponding with these, three factors appear to be at work: a seasonal factor tending to reversion of a temporary character; a reversion factor; and a mite factor; and it is usually extremely difficult to separate the two last, and to be quite sure whether fall in leaf vein number is due to the reversion factor only and not the mite factor, or whether it may be due to both.

The object of the present investigation, which was undertaken at the suggestion of Mr Lees, was to ascertain if there were any anatomical differences between reverted and normal plants, and if so, whether such differences could be correlated with the morphological evidences of the disease.

For this purpose a large number of normal and reverted plants were examined, and sections have been cut from every part of the plant. All the material used was obtained from the Agricultural and Horticultural Research Station, Long Ashton.

ROOT.

A very young root of the normal plant, before secondary thickening has begun, has not been examined, but a root with one ring of secondary growth shows clearly the primary wood in the centre, usually either triarch or tetrarch, with the smaller protoxylem elements towards the outside, and intermixed with parenchyma. The secondary wood consists of vessels and tracheids. In older roots the annual rings are very clearly marked. Aggregate medullary rays occur opposite the protoxylem elements. The phloem consists of multiseriate zones of soft bast without crystals, alternating with usually uniseriate rows containing crystals of calcium oxalate, the number of the latter increasing with the age of the root. There is a wide zone of phelloderm, and a narrow cork zone.

There is apparently very little difference between the normal and reverted roots. There is less parenchyma amongst the primary wood of the revert, and in the secondary wood, the vessels and tracheids are slightly smaller than in the normal, measuring 5–45μ in diameter as compared with 7–50μ in diameter in the normal. The medullary rays are wider in the reverted root measuring 7–28μ in width, as compared with 7–18μ in the normal; on the whole, therefore, the area of the medullary ray tissue in comparison with that of the wood is larger than in the normal, though this is not so definite as in the petiole and leaf (see below).

STEM.

In the young normal stem before secondary thickening takes place, there is a relatively large pith which is heterogeneous in character consisting of large and small-celled parenchyma. The primary xylem consists of rows of spiral and annular vessels measuring 18–36μ in diameter, between which are medullary rays one or two cells in width. The cambium is well marked, and the phloem consists of radial rows of large parenchymatous cells with no contents, alternating with small cells with dense contents and well-marked nuclei. Outside the pericycle and endodermis is the cortex composed of large parenchymatous cells, and two layers of epidermal cells.

In the old stem, cork formation takes place in the pericycle, and the bundles consist of wood, consisting of isolated vessels with scalariform thickening, and wood fibres which are prosenchymatous. The bast as in the root, consists of multiseriate zones of soft bast without crystals, alternating with usually uniseriate uninterrupted zones containing clustered crystals (De Bary (1)).

There is very little difference between the normal and reverted stem. The cuticle of the epidermal cells is somewhat thicker in the revert than in the normal. The wood cells are somewhat smaller in the revert, measuring 14·5–30μ in diameter as compared with 18–36μ in the normal, and have rather less definite contours. There are apparently no differences in the secondary tissues.

PETIOLE.

Three vascular strands pass into the petiole, and at the stem end these consist of wood vessels and prosenchyma, with medullary rays, usually uniseriate in character, and with dense contents. The phloem, as in the young stem, consists of radial rows of large parenchymatous cells which here are full of a dense gummy substance which may stain blue, red or yellow with haematoxylin and safranin; and small cells with protoplasmic contents and thinner walls. At the outer limit the phloem consists almost entirely of these large cells full of the dark staining substance. Further up towards the leaf, the walls of these cells are thickened, forming a zone of sclerenchyma. At the leaf end the sclerenchymatous zone is once more absent. The ground tissue consists of large thin-walled parenchymatous cells, somewhat heterogeneous in character.

The differences between the normal and reverted petiole are much more marked than in the stem (Figs. 1 and 2).

In the ground tissue and phloem the structure is the same but there is rather more of the red and yellow staining substances present in the revert and less of the blue. The medullary rays in the reverted petiole are usually two cells in breadth instead of one as in the normal; the cells are considerably larger, measuring 7–10μ in diameter as compared with 3·5–9μ in the normal. Their area compared with that of the wood is therefore larger in the reverted petiole than in the normal. They are less regularly arranged in the revert, and the cells like those of the phloem contain more of the red and yellow gummy substance.

The xylem elements in the reverted petiole, as might be expected from the condition of the medullary rays are also less regularly arranged. The vessels in the reverted petiole have less definite contours, and are considerably smaller than in the normal, measuring 15–20μ in diameter, while in the normal they are 20–25μ in diameter; the area of the wood as compared with that of the medullary rays is consequently smaller. The walls of the vessels are often thickened and stain bright red with safranin instead of the normal dull red; when this occurs, the middle lamella

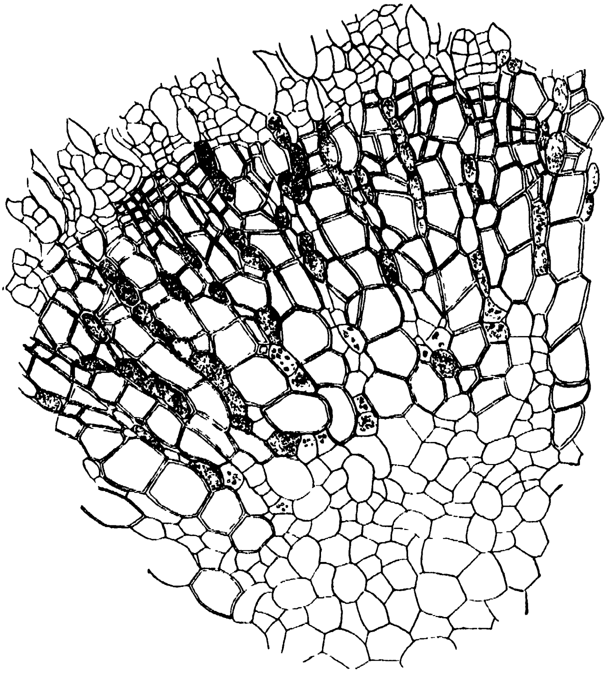

Fig. 1. Portion of bundle of petiole of a normal black currant. × 250.

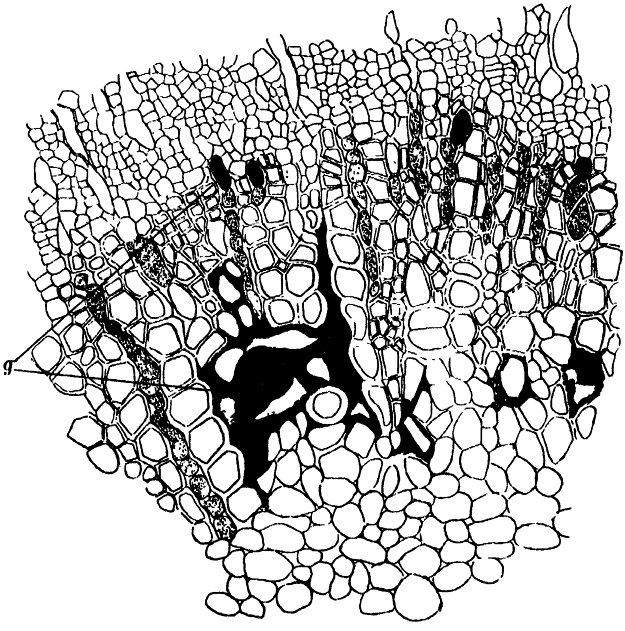

Fig. 2. Portion of bundle of petiole of a reverted black currant, showing irregularity of wood and accumulation of gum. × 250. g = gummy substance.

becomes very indistinct or may not be visible. A gummy substance which stains bright red with safranin, similar to that in the phloem, may be produced, completely blocking some of the cells or entirely destroying them. This resembles somewhat the formation of wound gum, but the substance produced in *Ribes* gives none of the reaction for wound gum. The substance occurs normally in all parts of the plant, and is probably similar to, or the same as, that excreted from the glands on the leaves and flowers. It is insoluble in water, alcohol and xylol; sulphuric acid and caustic potash apparently have no effect upon it, and it does not stain red with phloroglucin and hydrochloric acid. Apparently it tends to be produced in greater quantities in reverted than in normal plants.

LEAF.

The leaf of *Ribes* has a bifacial structure; the upper epidermis consists of large cells somewhat irregular in size, and the lower of smaller cells more regular in shape and size, and with stomata with small guard cells (Solereder (3)). The palisade tissue consists of two layers of cells. Below the bundles at the main and sub-main veins is a large-celled parenchyma; clustered crystals occur in some of the cells of this tissue, and also in certain cells of the palisade and spongy parenchyma.

The phloem of the bundle consists as in the petiole, at the outer limit of large parenchymatous cells full of the, here, blue-staining substance; and further in of radial rows of these large cells alternating with the smaller cells with protoplasmic contents. No sclerenchyma is developed in the phloem. The cambium zone is well marked, and the wood consists of vessels regularly arranged, alternating with medullary rays.

The differences between the normal and reverted leaves are, as might be expected, of the same order as those in the petiole but on the whole less well marked.

There is apparently no difference in the phloem, but the wood vessels in the normal are more regularly arranged, and have more definite contours than in the revert; moreover, they are larger in the normal, measuring 15–45μ in diameter (in the main vein) while in the revert they measure 12–35μ in diameter. There is on the whole less wood in the revert than in the normal. The area of the medullary ray tissue is correspondingly larger in the revert, the width of the ray being 7·5–15μ as compared with 5–12μ in the normal (Figs. 3 and 4).

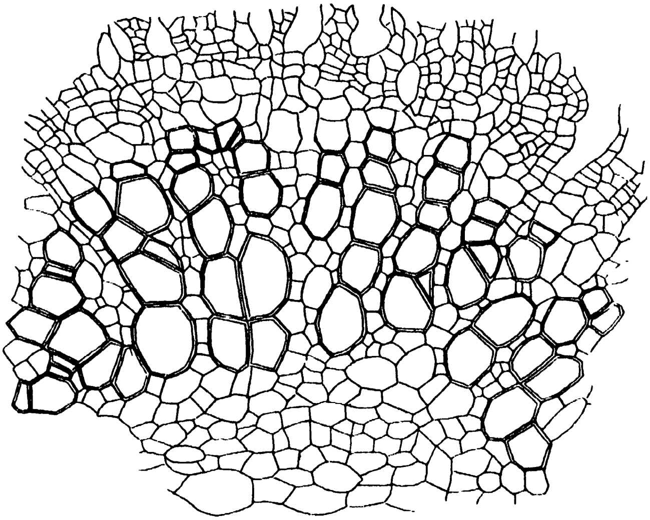

Fig. 3. Portion of bundle of leaf of a normal black currant. × 250.

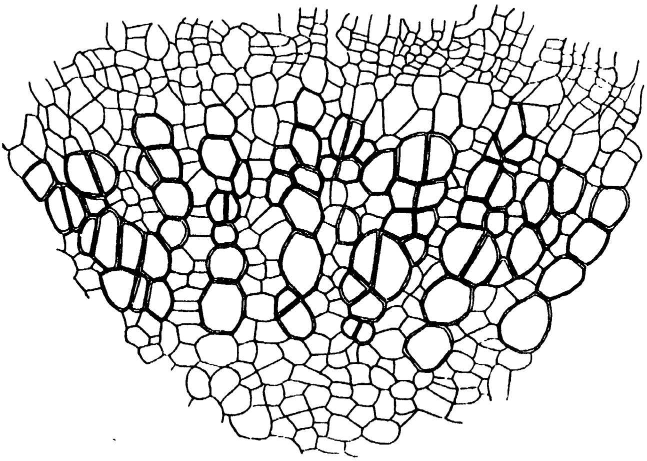

Fig. 4. Portion of bundle of leaf of a reverted black currant, showing irregularity of wood, smaller vessels and wider medullary rays. × 250.

PEDUNCLE AND PEDICEL.

The peduncle and pedicel have essentially the same structure as the young stem. The phloem has the same typical structure as in the stem petiole and leaf; the cambium zone is well defined, and the xylem consists of rows of vessels alternating with medullary rays, though these are not as definite as in the stem owing to the smaller size, and consequent reduction in the relative amounts of the various tissues.

Here again the differences between the normal and reverted petiole are of the same order as have been noted in the rest of the plant. The phloem in the reverted peduncle has considerably more of the red staining substance and less of the blue (cf. Petiole). The medullary ray tissue is larger in comparison with the wood, and the cells as in the phloem contain more red staining substance. In the xylem the cells are distinctly smaller in the revert, measuring 3·5–10·5 μ in diameter, while in the normal they are 3·5–18·0 μ in diameter. The area in comparison with the medullary ray tissue is smaller than in the normal, and the vessels are less regularly arranged.

FLOWER AND FRUIT.

The fruits of black currant plants suffering from reversion either do not form at all, the flowers themselves shrivelling up and dropping off; or the fruits may begin to form, in which case development is soon arrested and the half-formed berries shrivel and fall.

Sections through a normal unripe fruit show a large well-developed, and profusely branched vascular system. The wood vessels are large and numerous, and there is a considerable quantity of the blue and red staining substances in the phloem, and cortex surrounding the bundle. The ground tissue consists of large parenchymatous cells.

The ovary is large and well-developed and the ovules are normal. There is a layer of large cells containing the blue staining substance around each ovule; around the embryo-sac there is another layer of smaller cells containing either the blue, red or yellow staining substances; this layer widens into a cushion of cells at the antipodal end also containing the red or yellow staining substances. The nucellus in the earlier stages is well marked and healthy, and reproductive nuclei are visible in some cases (Pl. VII, fig. 5).

In the reverted plant, where formation of the fruit has begun, the condition of the fruit as shown by sections is very different. The vascular system is reduced and not so profusely branched; the wood vessels are smaller in size and fewer in number, and there is considerably less of the blue staining substance in the phloem.

The ovary is small and poorly developed, and the ovules reduced and hypertrophied. The layer of large cells surrounding each ovule is absent except in one or two cases—this is very striking. The nucellus in most cases is unhealthy and the cells are degenerating; no embryo-sac is developed and no reproductive nuclei (Pl. VII, fig. 6).

In some cases where the fruit has not begun to develop and the whole flower has dropped off, sections show that the vascular system is still more reduced and may even be absent altogether. An enormous amount of gummy substance may be produced at the base of the ovary, and in some cases completely filling all the ground tissue; what vascular system is present being blocked by this. The ovary is very reduced in size, and the ovules are aborted. The stamens are hypertrophied and the pollen grains arrested in development (Pl. VII, fig. 7).

GROWING POINT.

Sections were cut through the growing points of normal stems, reverted stems without big buds, and reverted stems with big buds.

No difference is apparent between the first two. In the third, however, as would be expected, the growing point is flattened, growth being in a lateral direction rather than a vertical, thus giving rise to a big bud.

GENERAL CONCLUSIONS AND SUMMARY.

No actual qualitative differences in structure have been observed between normal and reverted plants of *Ribes nigrum*; those that have been observed being rather quantitative, *i.e.* differences in degree only, *e.g.* amount of gum present, and variations in the amount of wood and medullary ray tissue.

Briefly the main differences showing to a greater or lesser extent practically all through the plant are a reduction in the amount of wood, and a consequent increase of medullary ray tissue, coupled with a tendency to produce more gum in the revert plant than in the normal.

On the whole, it is very difficult to correlate the morphological evidences of the disease with any of the anatomical differences observed, as the latter seem quite inadequate to account for the comparatively large morphological effect.

The general reduction in the amount of wood present, does not seem sufficient to account for the generally smaller and more unhealthy appearance of the revert bush; nor in the petiole even combined with a certain amount of blocking by gum, can it be held to account entirely for the typical reverted leaf, with fewer veins and reduced leaf surface. In the case of the fruit, although the vascular system is reduced, there seems no reason why some of the ovules at least should not develop. It is probable that there is some physiological factor at work causing hypertrophy and abortion.

My thanks are due to Mr A. H. Lees, who has supplied me with all material and given much valuable help and advice. I also thank Mr F. Summers and Professor O. V. Darbishire for their advice and interest in the work.

REFERENCES.

(1) De Bary (1884). *Comparative Anatomy of the Phanerogams and Ferns.* Translated by Bower and Scott. Oxford.

(2) Lees, A. H. (1922). Leaf Character in Reverted Black Currants. *Ann. App. Biol.* ix, 1, 49.

(3) Solereder (1908). *Anatomy of the Dicotyledons.* Translated by Boodle and Fritsch. Oxford.

EXPLANATION OF PLATE VII

Fig. 5. Transverse section of ovary of normal black currant. × 25.

Fig. 6. Transverse section of ovary of reverted black currant. × 25.

Fig. 7. Longitudinal section through flower of reverted black currant showing complete blockage by gum. × 25.

(*Received January 20th,* 1924.)

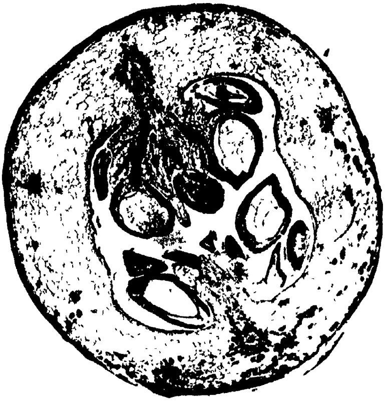

Fig. 5.

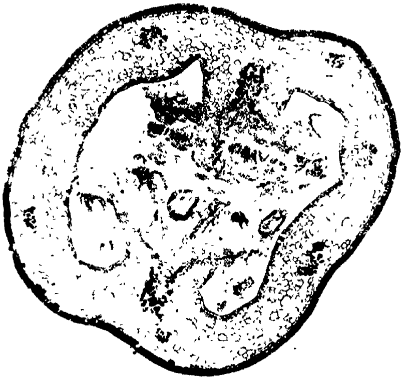

Fig. 6.

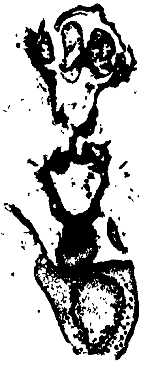

Fig. 7.

RIDLER.—Investigation of the Structure of Reverted Black Currants. (pp. 252—260.)

PROCEEDINGS OF THE ASSOCIATION

Pseudococcus sacchari Ckll. *and its Associates in Madeira.* By Dr MICHAEL GRABHAM, Quinto do Val, Madeira.

(*Read on* Dec. 7th, 1923.)

The invasion of the Egyptian sugar-cane plantations by the deadly Pseudococcus has led me, in answer to an appeal from the authorities in Cairo, to study and report upon the life-history and associations of the Coccid in Madeira where it is prevalent and unrestrained though negligible in effect on the sugar output owing apparently to the cultivation of a variety of cane overflowing with vitality and equal to a severe strain.

The cultivation of sugar-cane in Madeira seems to have commenced early in the fifteenth century, soon after the sailors of Portugal had stumbled upon the Archipelago in the days of Prince Henry the Navigator. This Prince had an English mother and his life was devoted to the expansion of his country and colonial development.

The cane came from Sicily and after the westward enterprise of Columbus seems soon to have spread from Madeira both to Canary and the West Indies.

Preference was always given to the cultivation of the vine, for it was soon realised that sugar could be grown in any warm country whereas the specific quality of Madeira wine was limited and local.

From time to time a blight or other cause would obliterate the vine, and sugar-cane would become predominant: and again in like manner the cane would periodically fail.

Thus in regard to the vine, the vineyards were completely destroyed in 1852 by the fungoid *Oilium Tacteri*, and again in 1877 by the underground apterous Aphis *Phylloxera vastatrix.*

But in these days the maintenance and favour of the sugar-cane have been stabilised in Madeira by preferential regulations at Lisbon.

The Bourbon variety of cane had been cultivated in Madeira from time immemorial until the year 1877, when a fungus, probably *Aspergillus fulvus*, was introduced and spreading along over terraces destroyed every cane by intrusion into the stem substance where it converted juice and sugar into a thick mucilaginous nauseous ooze. The fungus itself died with the cane and has not since reappeared.

No attempt was made to replant sugar-cane after that dire destruction and the ground was occupied for several years by the vine, sweet potato and general food produce.

The *Pseudococcus sacchari* was sparsely present before the arrival of the *Aspergillus* fungus and I think was largely responsible for the introduction of the fungus through its many punctures into the substance of the sugar-cane already weak from prolonged cultivation and loss of protective power.

Strenuous efforts were now made to find a cane new to the locality and of greater resisting power, and the Yuba cane from Natal was adopted and has since thriven and yielded well.

The Pseudococcus either survived the Bourbon cane destruction in some manner or was reintroduced. At all events it appeared at once as the Yuba cane spread in the same sparse presence as in the years anterior to the coming of the *Aspergillus fulvus.*

The Madeira terraces were bare of sugar-cane for quite three years, thus giving ample time for the mealy bug to die out, if not otherwise sheltered.

For a while I felt fairly certain that a *modus vivendi* had been provided by the elegant tomato tree, *Cyphomandra betacea,* and also by the shrubby *Guatamala solanum*, with the result that both these plants of high economic value have practically disappeared from this island: but my experiments this year go far to acquit the bug of this evil, for though I have transplanted whole colonies of it with the greatest care on a thin paring of cane into the seclusion of banana or strelitzia sheathing, into the fleshy stem of dichorisandra, or the attractive juices of the custard apple, I have in no single instance succeeded in persuading the noxious creature to thrive or even survive away from the sugar-cane, and in all this I am fully confirmed by the experience of Mr Ward, the entomologist of the Egyptian Government.

I am just now watching a badly infested plot from which my men have outrooted the cane and without further preparation planted the sweet potato convolvulus in trenches filled with sugar-cane debris swarming with Pseudococcus. The convolvulus is all rooted and growing rapidly, but though the plant is badly subject to lecanium scale, not a single individual of the sugar mealy bug has appeared. I have found a spray of bitter quassia to be distinctly effective against the bug in an early stage.

The Argentine ant found its way to Madeira 30 years ago when the Yuba cane was already well-established, and the response of the Pseudococcus to the fostering influence of the new pest became at once manifest in the startling increase and activity of the cane parasite. The ground was honeycombed with formicaries among the cane roots, and during the fortnightly irrigation of the cane the legs of my men were black with the myriads of ants seeking this shelter from the running stream of water among their nests.

I may mention, in illustration of the fecundity of the Argentine Iridomermyx, that I had destroyed 40,500 ants from a single lemon tree without making a tangible impression upon their numbers.

The articles of partnership between this ant and the Coccidae I have never mastered, though Madeira abounds with opportunity for study. The ant certainly provokes or augments the flow of honey dew and also ministers obviously to the welfare and overwhelming fertility of the bug. Without the ant the Pseudococcus and its kindred languish and wander disconcerted: and though they can run alone without the ant, they dwindle in numbers and are no longer formidable in their depredations.

The Pseudococcus is now found abundantly in every cane plantation in Madeira: you may look for it with confidence under every leaf sheath which has separated from the growing stem sufficiently for the young migrating female to enter: and a colony at the node where they congregate will number from twenty to several hundreds of individuals in every stage of growth. All of them are females and reproduce parthenogenetically in fabulous numbers, and a full-grown cane will support very many well-established and thriving communities in countless myriads.

The bug feeds on the cane stem itself, attaching itself very rarely to the leaf sheath. From every colony a stream of honey dew laden ants constantly issues, many of them carrying besides any debris or gummy exudation associated with the mealy bug. The parasitic community is hence kept clean and free from the adhesive impediment to stripping described in Egypt.

The sugar mealy bug requires shelter, and hence when the lower cane leaves dry and retreat from the stem the younger members of the community migrate upwards—naked and not ashamed, to find in more succulent regions an opening to a more sheltered and nutritious node.

You may see on any mature cane tiny wandering females searching for the first sign of a new leaf parting from the stem, ready to enter into a hardly perceptible crevice. It is said that the ant itself transplants the young ones, but I have never seen this and the free mobility of the sugar mealy bug is sufficient without such assistance. But the ant follows the bug as soon as there is room enough to pass within the sheath.

A shower of rain will fill every sheath with water and drown every ant which has not had time to escape, but if you tear the leaf sheath asunder you will find every bug alive and undisturbed by its immersion for many days.

The older ones remain below and there secrete more copiously their protecting mealy threads and waxy exudations. But this species is sparing of its cottony secretion even when exposed.

The male Pseudococcus I have only seen once, viz. in March of the present year on an exposed position of the cane stem. He is probably born late in the life of his virgin parent and seems to pass his ephemeral career in the society of mature or even aged associates whose waning fertility may require sexual recuperation.

He is troubled with no alimentary developments, not even a mouth has been given him, and he is thus able to devote undivided attention to the matrimonial attractions of his exacting mates.

Thus infested and ministering to the sustenance of countless parasites and overflowing moreover to the supply of an insatiable stream of ants who night and day ceaselessly milk their plump protégés, the Yube cane in Madeira has survived in unabating vigour quite 30 years, showing even yet no sign of deterioration either in vitality or crystallisable sugar yield. Such a vitality is truly miraculous and inexplicable. No control measures are undertaken or thought of: the cane is cultivated, manured and cut year after year—sometimes for 20 years without replanting—and Pseudococcus and ant are left to work out their own salvation unmolested.

All sorts of supplementary crops are interpolated and gaps in every plantation are filled with Indian corn, beans and sweet potato plants, and all these thrive until choked out of existence by the overwhelming cane growth which, with a girth hardly exceeding that of the *Arundo Donax*, reaches a height of 8–10 feet in rank superiority and in spite of the drain it suffers from myriads of a relentless parasite.

The *Pseudococcus sacchari* has no restraining enemy in Madeira. It is jealously protected and fostered by the Argentine ant as I have stated, and in the presence of so vigilant a participant of its stolen goods no chance is given to any hungry intruder.

My friend, Professor Cockerell of Colorado, an accomplished naturalist and keen observer, speaks of finding signs of a dipterous parasite among the debris of the cane mealy bug, but I have found no trace of such an enemy, neither has any appeared in the badly infested cane specimens which I have sent to Egypt for comparison.

Formerly we had plenty of lady-birds, both of the common European species and locally specialised forms, and yellow patches of their eggs were freely found on any coccus infested twig. But the ant now destroys every such interloper whether coccinella or kindred scymnus. Hence, so long as this pernicious ant exercises a strict supervision, the introduction of an efficient coccinellid, such as a Cryptolaemas, in some form would be an expensive futility.

Mr Ward, in his excellent account of the Pseudococcus in Egypt, regards the rat as a potent enemy to the cane parasite and speaks of holes or windows torn in the leaf sheath in search of the bug. In Madeira this is not so, no such windows appear, neither does the rat, which is here destructive and abundant, molest the bug when freely exposed by the retreating sheath. But the rat, if undisturbed, will scoop out the luscious substance of the lower part of the cane for 2 or 3 feet and thus leave it to perish.

Of possible fungoid enemies I am aware of none exercising any control of economic importance.

The Yellow Aspergillus obliterated the sugar industry, as I have stated, having, as I believe, been introduced into the substance of the cane through the perforations inflicted by the long sucking tubes of the mealy bug. The red fungoid stain I show you seems to be of a kindred nature, but this, though generally prevalent in the leaf sheath, has not been known to penetrate the cane itself. To verify this, I have carefully grafted fungus infected sheathing into the cane substance, and paring the surface of both cane and sheath, I have secured both together without obtaining the faintest staining of the cane tissue. And I know of no other fungus in association with the cane or mealy bug.

We have thus in Madeira a variety of cane which in 30 years has suffered no impairment though beset with exhausting parasites. Our sugar is yielded in full volume and of the attractive quality of the specimen before me.

The object of this paper is to contrast the local conditions at Madeira concerning the sugar-cane and its Pseudococcus parasite with those reported from Egypt, and to elicit what is known of the subject elsewhere.

The Work of the Fisheries Laboratory, Lowestoft[1]. By E. S. RUSSELL, M.A., D.Sc., Director of Fishery Investigations, Ministry of Agriculture and Fisheries.

The laboratory consists of a large detached building on the South Esplanade standing in its own grounds, and directly overlooking the sea. Ample accommodation is provided for the staff and there is plenty of light and air. The laboratory is now reasonably well-equipped with apparatus and there is a useful library which is being gradually improved. Our chief lack at present is a tank-room with a circulation of sea-water. This is a very serious drawback, but it is hoped in the future, when the country is more prosperous and money for research is not so tight, to have a proper series of tanks constructed and housed in a tank-room to be built in the grounds. A number of small table tanks have been provided in one of the basement rooms and an aerating system has been installed, modelled on that in use at some of the Danish laboratories.

Our research ship the *George Bligh* is an ex-Admiralty trawler of the Lord Mersey type, built in 1917. Certain alterations have been made to adapt her for fishery research and accommodation has been provided for the naturalist staff forward, in part of the former fishhold, and a laboratory under the bridge in what used to be the skipper's cabin. Her fishing equipment is, generally speaking, that of an Iceland trawler. She has a 1000 fathom winch enabling her to trawl in 200–300 fathoms. Special equipment includes a steam Lucas sounding machine and a special light winch mounted on a platform in front of the bridge which has proved very useful for working small gear.

Her maiden voyage as a research ship was made in April 1921 to the Lousy Bank, which is a little-known Bank lying between Rockall and Färoe. Very bad weather was experienced on this trip, which was unsatisfactory so far as finding fish was concerned, but she behaved extremely well and has proved to be an unusually steady and comfortable ship. A small bank discovered near Rockall has been named George Bligh Bank.

We have at present no small vessel for inshore investigations attached to the laboratory[2] but this lack has been partially remedied by the installation of a motor in one of the ship's lifeboats, this being detached temporarily from the ship, for a day or two at a time, for work in such places as the Wash and the Thames, or along the Suffolk coast.

As regards the kind of work we have been doing and plan to carry on and develop in the future, the main energies of the staff both under the M.B.A. and under the Ministry in London were devoted to the study of the plaice. By 1914 the plaice investigations had been in the main brought to a conclusion; the broad outlines of the natural history of the plaice in the southern North Sea were pretty thoroughly known, and definite proposals for the protection of the plaice, based upon the research work of all the participating countries, had been put forward by the International Council for the Exploration of the Sea. Then came the war, which brought about a very striking and very instructive change in the size and distribution of the plaice in the North Sea. There were more plaice than before, and they were markedly larger and older than the pre-war plaice. The growth-rate had diminished and there was a partial reversion to the state typical of a virgin ground. This interesting development demanded investigation, the more so that certain continental countries revived the proposal for an international size limit. A year's intensive study of the plaice was agreed upon at the I.C. meeting in March 1920 and duly carried out. I do not propose to enter into the methods and results of that year's work, interesting as the results were[3]. Plaice work on a big scale was definitely brought to an end in April 1921, and plans made to begin the study of other fish of equal importance. At the I.C. meeting in July 1921, we undertook to devote special attention to the cod and the herring, and all our work since that date has been concentrated directly or indirectly on cod and herring problems.

[1] Read on Nov. 10th, 1922, revised and brought up to date (Dec. 1923).

[2] One has recently (Jan. 1924) been acquired for work in connection with local by-laws.

[3] See J. O. Borley and others, "The Plaice Fishery and the War," *Fishery Investigations*, Ser. II, vol. v, no. 3, 1923.

The first line of investigation which we started was the quantitative study of the bottom-fauna in relation particularly to the food of cod, haddock and plaice. Many of you are no doubt familiar with the pioneer work of Dr Petersen in the Belts and the Cattegat carried out by means of his bottom sampler, which enabled him to map out the communities of bottom living animals in those waters and to estimate the approximate amount of available fish food per unit of area. We have been successful in applying this method in the open waters of the North Sea.

With the kind assistance of Prof. Hjort, an arrangement was made with Dr Petersen to send his assistant Dr Blegvad to Lowestoft to put us wise to the method of using the bottom sampler and the technique of working up the results. A special cruise was run by the *George Bligh* from Lowestoft to Esbjerg in July 1921, when 20 stations were worked. Dr Blegvad, who took part in this cruise, has written an account of it which has been published by the Zoological Society[1]. The main results of this preliminary survey were to show that the bottom sampler could be used without much difficulty from a biggish ship in open waters, and that several of the communities distinguished by Petersen were to be found in the North Sea—notably the *Venus* community and the *Echinocardium-filiformis* community. Having thus been shown the way, we have since devoted a good deal of time to work with the bottom sampler. It was found best to use a larger form of grab than that generally employed by Petersen, as the additional weight made it delve deeper into the bottom and rendered it less liable to overturn in bad weather when the drift of the ship is apt to make the haul an oblique one. The grab we use has an area of 0·2 metre sq. or roughly 2 sq. ft.

As it was clearly impossible to sample the whole North Sea adequately we concentrated first on an area on the Dogger Bank about 30 miles square, with the idea of working this thoroughly two or three times a year. Casual survey work is done in addition on ordinary cruises with a view to mapping out in a general way the distribution of the communities in the North Sea. The Dogger Bank area was selected for intensive work because of the proposal (which at one time seemed likely to come to fruition) to transplant small plaice on a commercial scale from the Continental inshore waters to the Dogger Bank. It seemed desirable to make some sort of estimate of the amount of plaice food present on the Bank, in order to be sure that there would be sufficient available for the new population.

The results of an intensive survey of the selected area on three cruises carried out in the autumn of 1921 were instructive. The fauna was found to be very patchy. In general it was not particularly rich, but here and there very rich patches of *Mactra* (*Spisula*) *subtruncata* were found. Thus at one station over 200 large specimens were taken in one haul from an area of 2 sq. ft., and at another over 800 small specimens of the same shellfish. As this form is greatly sought after by the plaice the result is an important one from the fishery point of view.

The fauna belonged generally to the *Venus* community with a tendency to Deep *Venus*, but the correspondence with Petersen's communities was not a close one and it may be necessary to found a new community for this Dogger area or perhaps to revise the whole community idea in the light of our recent results.

Among the interesting forms found by the use of the grab were *Amphioxus*, which we found to be common in patches of gravel on the Dogger, and a specimen of *Glossobalanus*[2] taken off the Northumberland coast. It is hoped also by seasonal work to get some useful data as to the rate of growth of the commoner forms on the Bank.

During 1922 we continued the investigation, extending it to the whole Dogger Bank area, on a more intensive scale than before, paying special attention to determining the extent of the rich patches.

A particularly successful cruise was carried out in October when the greater part of the Dogger Bank was surveyed, 150 stations being worked at 5-mile intervals, two hauls at each. The result has enabled us to map out the positions and extent of several large patches both of *Mactra stultorum* and of *Spisula subtruncata*, the patches of the latter being several miles in length and breadth, and showing a remarkable graduation from small numbers of these shellfish at the edges to very high numbers at the centre of the patch, the range on a typical patch being from 5 to 8000 per metre square. Much information is being accumulated as to the rate of growth, time of spawning and death-rate of these shellfish. A preliminary report on the results up to February 1923 has been published by Mr F. M. Davis[1] and the work is still going on.

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[1] *P.Z.S.* 1922, pp. 27–32.

[2] See A. Meek, *Q.J.M.S.* LXVI, 1922, pp. 579–94.

In connection with these investigations a type collection of the North Sea fauna is being formed and contains now 400–500 forms. The stomach contents of many fish, particularly the cod, have been examined.

Another main line of research has been the study of the early stages of the herring. We have broken away from the orthodox plan of concentrating attention on the age and maturity of the adult herring and have given most of our time to a search for the spawning grounds and the larvae and post-larval forms. Surprisingly little is known about the spawning grounds of the herring in the North Sea or about its history during the first two or three years of its life, and we are trying to fill up this gap so far as the English area of investigations is concerned. We began by making a thorough search for herring spawn in certain areas, off the Northumberland coast mainly, and on the west and south edge of the Dogger, where it is known, principally through the landings of spawny haddocks, *i.e.* haddock gorged with herring spawn, that the herring do spawn in August–October. The search for the spawn itself, carried out by all forms of bottom gear, was a complete failure, either because the spawning was unusually restricted in amount or because we failed to find the patches. We found it much more profitable to go for the newly-hatched larvae and the post-larval forms, and with this object in view several lines of stations were worked repeatedly in 1921 and 1922 over the southern North Sea right into the Heligoland Bight, in the Wash, the Thames and the eastern part of the Channel.

Very rich material for study has thus been obtained, the principal gear used being the Petersen young fish trawl.

Though the results have not yet been fully worked up it is already evident that there is a spawning ground off the Lincolnshire coast which no doubt contributes largely to the rich shoals of young herring known to frequent the Wash. Other spawning areas, already partly known, were discovered off the Northumberland coast and on the west edge of the Dogger. Large catches of post-larval herring—up to about 1000 in a half-hour haul of the Petersen trawl—have been made also at certain places in the Southern Bight, and the eastern Channel stations have also proved productive. A concentration in shallow inshore waters appears to take place at quite an early stage in the herring's life-history. It is hoped by continuing this line of investigation to map out the chief spawning areas and trace the passive distribution of the early stages. One significant and rather troublesome fact emerged from a special study carried out in 1921 on the variation in abundance of young herring. The ship was anchored for 24 hours and four sets of hauls by the Petersen trawl were taken at intervals. There was found to be a very big variation in the quantity of post-larval herring caught, a variation due not to vertical movements in the water but apparently to shoaling. This shows that one must be cautious about drawing quantitative conclusions from the catches made of the post-larval herring, even under an inch or so in length.

I come now to an interesting piece of special work on the herring which we carried out in 1921.

As you probably all know, the herring fishery in 1921 on the East Coast of England was a comparative failure. The fish were not found in their usual abundance; they were generally late in arriving at their usual haunts and they were ill-nourished. It had already been shown by the Scottish investigators that an abnormally large influx of Atlantic water had taken place round the north of Scotland into the North Sea in 1920 and 1921 and it was suggested that some connection existed between these abnormal hydrographical conditions and the scarcity of herring. We therefore arranged a cruise in October 1921 through the chief herring areas and across the

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[1] *Fishery Investigations*, Ser. II, vol. VI, no. 2, 1923.

North Sea north of the Dogger as far as the Great Fisher Bank. Hydrographical and plankton samples were taken at frequent intervals and the hydrographical results demonstrated quite clearly the presence on the bottom north of the Dogger and also in the gut between the Dogger and the coast of a thick layer of Atlantic water extending farther south than usual[1]. The plankton results at certain stations, particularly between the Dogger and the coast, gave an apt confirmation of this result, for large quantities of the Atlantic pteropod *Limacina lesueuri* occurred together with the more usual *Limacina retroversa*, also a species of *Peraclis* and the Atlantic radiolarian *Phyllostaurus quadrifolius*[2].

Additional evidence was found in the temperature and salinity records from various lightships and steamship routes for 1921 of the encroachment of the Atlantic water[1]. What the relation between this unusual state of the North Sea and the scarcity of herrings is, cannot be stated, but it seems probable that there *is* a relation and, bearing in mind the poor nutritive quality of the fish, it seems likely that this relation is a mediate one through the food supply. In this connection certain results obtained in 1920–1 from a quantitative study of the plankton associated with plaice eggs and larvae in the Southern Bight may be significant. It was found that the plankton was richest, not in the tongue of Atlantic water which pushes up into the North Sea from the Channel during the winter, but at the sides of it, in the coastal water, particularly on the Dutch side[3].

In any case it is obviously important for us to make a close study of the food of the herring and of the origin of that food. A beginning was made with this line of investigation during 1922 and it was actively prosecuted during 1923. Study of the very young stages has yielded results confirmatory of Miss Lebour's work at Plymouth. The very young ones, even before they lose the yolk sac, appear to select their food from the plankton and show a very marked preference for *Pseudocalanus*. Larger ones go for *Temora* and the herring of whitebait size so far as studied seem to specialise on *Eurytemora*. A good deal has also been done on the food of the adult herring. It has proved necessary to get quite fresh stomachs, as digestion appears to be rapid and to continue after the death of the fish, but samples are being obtained for us on drifters by the crews. One sample, taken by the *George Bligh*, had been feeding largely on cod and plaice eggs.

Though we are concentrating rather on the early stages of the herring's life-history the study of age and maturity is by no means being neglected. Many samples of commercial herring have been examined, and uniformity of method has been achieved by sending a member of the staff to Norway to study under Prof. Hjort and Mr Einar Lea.

Before leaving the subject of herring investigations I might mention that a member of the staff made two or three flights in a seaplane off Lowestoft to investigate the possibility of spotting herring shoals from the air. The experiments were carried out in co-operation with the Air Ministry during the height of the herring season. The flights were quite successful and not marked by any untoward incident but the results were definitely negative, as it proved impossible to see the herring owing to the water being thick with suspended sand and mud. Better results have been obtained by the French in the clear waters off the Brittany coast, especially by the use of an airship. Further flights were arranged off the Cornish coast, but were unsuccessful owing to bad weather.

We come now to the cod. A large amount of data is being collected as to the size of the cod taken by the trawl through the agency of a staff of fish measurers who make trips constantly on commercial trawlers and measure large samples of the catch. The plaice and the haddock are also measured, and the haddock measurements are handed over to Scotland under an agreement by which Scotland tackles the haddock while we specialise on cod.

Special work on the cod, apart from the routine fish-measuring and the bottom fauna work, has consisted hitherto principally in a series of marking experiments carried out, first at Flamborough on the Yorkshire coast by a member of the staff working in the local cobles, and later by the research vessel. About 750 cod, mostly large ones, have been marked, and the returns have been good. A large number of cod have been examined on the market at Bridlington and at sea as to their state of maturity, and much work has been done on the food. Statistical studies have also yielded valuable results[1].

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[1] See J. R. Lumby, International Council, *Public. de Circonstance*, no. 80, 1923.

[2] See A. C. Hardy, *Pub. de Circ.* no. 78, 1923.

[3] R. E. Savage, *Fishery Investigations*, Ser. II, vol. v, no. 6, 1923.

I come now to the last main line of work carried on, namely, hydrography. My policy here has been, for reasons which will appear later, to carry out a small regular programme of general hydrography on lines suggested by the Hydrographical Section of the I.C. and to continue in a revised form the system previously inaugurated of obtaining surface observations of temperature and salinity on steamship routes.

The general hydrographical programme which we put into operation at the beginning of 1922 at the instance of the I.C. comprises (1) current observations by means of the Jacobsen meter three times daily at several depths on the Varne and Smith's Knoll Light Vessels, (2) daily observations at the same Light Vessels of surface salinity and temperature. In addition the observations hitherto carried out at the Seven Stone Lt. Vl are being continued as well as those made on the various cross Channel routes and on one route across the North Sea. New lines of steamship observations have been started in the Irish Sea and across the southern part of the North Atlantic. On the other hand, regular sampling from the Research Vessel has for the most part been discontinued, as it has been found that such observations are too sporadic both in space and time to be really valuable. Observations of temperature and salinity at different depths are however made from time to time in direct relation to specific fishery problems. We have arranged also to carry out every year two or more hydrographical cruises along the general line Tyne to Naze of Norway, with the purpose of getting some idea of the spread of Atlantic water from the North, and these cruises have been regularly carried out. Much work is also being done on currents by means of drift bottle experiments.

Having given you an idea of the sort of work we are doing at Lowestoft I propose now to say a little about the guiding principle on which it is all based. This guiding principle is a very simple one. It is that the chief aim of fishery research should be to gain a complete and many-sided knowledge of the natural history of all the fish of economic importance, a knowledge such that rational measures can be taken to conserve and possibly in certain cases to increase the supply available for food. All the different lines of fishery work, as for instance plankton investigations, bottom fauna work, statistics, fish measuring, hydrography, ought to be concentrated on the study of the particular species of fish which is being investigated. The fish ought to be the centre of the investigation and all lines of work should be definitely and closely related to this central problem. Thus investigation of the plankton should not be carried out, by a Fisheries Department at least, primarily for its own sake, but from the point of view of its forming the food at some period or other of the particular species of fish being studied. We should make a special study of those plankton species which are in this way important, investigating their origin, distribution, conditions of development and growth.

In the same way, in studying the bottom fauna, we should concentrate attention on the species which are important as fish food, try to estimate the quantity of this fish food, study it in relation to the stomach contents of the fish and its habits of feeding. It should not be our primary aim, as a Fishery Department, to chart out the distribution of all the bottom living forms, however interesting this would be from a purely zoological point of view. From the study of the fish food whether planktonic or benthonic we should pass outwards to a study of the organisms upon which the fish food feeds, and thence to a study of the physico-chemical conditions influencing production in the sea. Our procedure should be to start from the fish and work outwards to the general conditions rather than the other way round. The fish and its history should be the guiding thread running through all our work and giving it significance and coherence.

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[1] See Michael Graham, *Fishery Investigations*, Ser. II, vol. VI, no. 6, 1924.

I am aware that this policy of concentrating everything round the fish and leaving out to some extent the general aspects of oceanography as a whole, has its dangers. It is always unwise to concentrate overmuch in obtaining immediate economic results at the cost perhaps of basing these upon insufficient knowledge of the very complex conditions obtaining in the sea. But I am looking at the question primarily from the point of view of the fishery biologist who is bound to keep economic questions well in the forefront and to try and get results definitely of use to the trade and to the nation.

There are other bodies at work both in this country and in others whose function it is to study oceanographical problems and problems of general biology and physiology from a purely scientific point of view Witness the many excellent papers produced by the Marine Biological Association in this country and the work which used to be turned out at Naples. All that is available for study and instruction, and to give pointers for economic research.

Then again, I must admit that this intensive scheme of fishery research rests upon the great and valuable work done in the past upon general lines. We know roughly the general distribution of plankton in our seas, the broad outlines of the distribution and variation of temperature and salinity, the main movements of the water, and without this knowledge our specialised fishery work could not be fitted into its proper place in the general science of the sea. I submit, however, that we do know in broad outline what is happening in our waters and that we can accordingly fit our fishery work into the general scheme of things and see it in its proper relations to oceanography as a whole.

Another reason exists—a not unimportant one at the present moment—why fishery work should be concentrated upon its own special problems and our forces not dispersed too widely on general lines. It is that at present the Fisheries Department has not the means—either in material or in personnel—to do oceanographical work on the grand scale.

We have a staff which is just sufficient to carry out the programme laid down on cod and herring and we have only one ship. This ship is fully occupied in the southern half of the North Sea in biological work. We cannot at the same time tackle any elaborate investigations in other waters, notably in the south-west area off the mouth of the Channel so important for the study of mackerel and hake. Accordingly we have to cut our coat according to our cloth and limit ourselves to doing such work as we can really carry out.

REVIEW

British Hymenoptera. By A. S. BUCKHURST, A.R.C.S., D.I.C., L. N. STANILAND, A.R.C.S., D.I.C. and E. B. WATSON, A.R.C.S., D.I.C. With an Introduction by H. M. LEFROY, M.A., Professor of Entomology at the Imperial College of Science. Pp. 48. 8 plates and 16 text-figures. London, Edward Arnold & Co., 1923. Price 9*s*. net.

The writers of this volume collaborated with Prof. Lefroy in his *Manual of Entomology* reviewed in the last number of the *Annals*, and figures from that book are used for the illustration of this, supplemented by sixty-two reproductions of photographs which are, for the most part, excellently clear. The text consists of brief accounts of the tribes and families of the Hymenoptera with special reference to the commoner British species, which the entomological student is likely to meet, as he starts to make a working collection or to observe the ways of living insects. The general introduction to the order lacks any reference to the nature of the jaws, and the treatment of other important structural features, such as the wings, is very meagre. Despite its small extent the book contains useful information, but the combination of wide pages and large type has resulted in a selling-price that seems somewhat excessive for a work of such restricted scope.

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THE RELATION BETWEEN PURE AND APPLIED BIOLOGY

BY PROFESSOR E. B. POULTON, M.A., D.SC., LL.D., F.R.S.

BEING THE ADDRESS OF THE RETIRING PRESIDENT OF THE ASSOCIATION, DELIVERED JANUARY 18TH, 1924.

AT the end of my term of office it is a pleasing duty to thank the officers of the Association who have made my work so light, the readers of papers from whom we have learned so much of interest and importance, and those who have contributed to the discussions which have given an added vitality to our meetings. It has been most inspiring to see such numbers of students coming together month after month to listen and I have no doubt to criticise; and this in the early afternoon, a time which for many is held to be sacred to the cult of the open air.

I have all through looked forward to the pleasure and privilege of thanking those who have made my term of office so easy and so interesting, and of expressing the confident hope that the same kind help would be extended to my successor. But when it comes to a Presidential address, that is a different matter. An address if required is a chief, probably the chief, responsibility of the position. I am a very busy man and the work seems to increase as I become older. Furthermore I have had to deliver many addresses. It is a good thing that, from time to time, a man should be induced to look at his subject from a new point of view, and I do not agree with an opinion recently expressed by a senior member of a scientific society, as the anniversary meeting was approaching—that "the whole thing is a waste of time: the President doesn't want to give an address and nobody wants to listen to it!"

A Presidential address is I believe a good thing, but a man should not be expected to give many. Suitable subjects are not very numerous, and, if treated seriously, all of them demand much time and thought. A man owes it to himself, or as I prefer to say, to those who look to him for guidance, not to spend too much time in the preparation of addresses on the more general aspects of his subject. Therefore, when honoured with the invitation, I asked whether an address was necessary and received the assurance that it was not required. I hope that this will continue to be your tradition. We have many scientific societies in this country and your Presidents will often be men who have previously served elsewhere and some of them may find a real difficulty in again accepting a position which carries this responsibility.

In spite of the assurance given to me I am delivering a brief address in compliance with the wishes of our late Secretary, Dr W. B. Brierley, to whose efficiency and energy we owe so much. He was so persuasive that I felt compelled to compromise and agree to do as he wished, not however twice, but at the end of my term of office. It is the kind of thing one undertakes to do a year-and-a-half ahead—a distant future when the pressure of work will surely be relieved! That is the dream, but presently comes the awakening. It seems to be a law of human nature that as the fatal day approaches and time for thought is urgently required, work of all kinds, work which cannot be put off, steadily accumulates. Therefore today I can only offer you a spoken and not a written address. I hope however that this is not all loss, and that you will agree that speech face to face has some advantages over the more rounded written sentences more or less hidden behind a paper screen.

The revenge I might have taken is denied me. If Dr Brierley had remained in office I might perhaps have followed the example of an eminent President of a scientific society and have written a very polite letter to the Secretary asking him to give me his kind help by preparing notes on the recent advances in our science, and when the notes were received, I might have acted as he did and returned them to the Secretary with the most grateful thanks but asking if he would add to my indebtedness by throwing them into the form of an address!

It is of interest to compare for a moment Applied Biology with Applied Physics and Chemistry. Our friends sometimes take a rather superior tone in comparing their sciences with ours. I remember a Physicist friend who, when it was maintained that it would be useful to have some common term to include Physics and Chemistry just as Biology includes both Zoology and Botany, replied at once "Oh! but we have such a term—Science!" But there is reason to fear that the share in science which my friend allotted to Chemistry was very different from that which he claimed for his own subject. It was a haughty Physicist who defined Chemistry as "The dirty part of Physics and the clean part of Biology!"

In Applied Science, however, Biology has a great advantage over Physics and Chemistry—an advantage which is especially great in the department of learning with which this Association deals. Every problem concerned with living beings compels the student to search in many directions, to seek all possible help in the widest field, and generally to invoke the assistance of the haughty sciences themselves. The simplest living being is infinitely complex and enters into an infinitely complex indirect relationship with the world around it. The attempt to unravel a part of this entangled web is not only broadening and strengthening to the intellectual faculties but also to the social side of human nature. On other occasions I have dwelt upon the broadening influence of an intelligently directed specialisation, but this aspect is so often neglected that I do not hesitate to speak of it again. The infinitely complex problem set before the student of Applied Biology compels him to seek help from other specialists. His own work shows him the uselessness of an opinion founded on superficial study, and he realises that the rapid glance of one who knows is generally worth far more than a prolonged examination by one who is without an intimate knowledge of the subject. He is thus led to pursue to the uttermost his own researches in his own part of the great field without any inclination to encroach on the sections where others are at work—to give help and to receive help, to take a keen and friendly interest in the investigations going on around him and to welcome the same interest in his own.

Specialisation pursued in this spirit, so far from being narrow and narrowing, is broadening as an education and broadening as a social force; for it leads to mutual appreciation instead of mutual jealousy. It is a spirit very different from that of a not distant past symbolised by Professor Ptthmllnsprts in *The Water-Babies*. Only recently I have come to realise, thanks to Stephen Paget in *I have reason to believe*, that the weird name is "Put them all in spirits" with the vowels left out! Well, you remember that Professor Ptthmllnsprts was said to be like a cock-robin, because "when anyone else found a curious worm, he would hop round them, and peck them, and set up his tail, and bristle up his feathers, just as a cock-robin would; and declare that he found the worm first; and that it was his worm; and, if not, that then it was not a worm at all."

But even in those years of fierce rivalries which seem so far off and are really so near, we find the highest of all examples of mutual sympathy and appreciation in the two great men who, on opposite sides of the world, hit upon the same discovery. On July 1, 1908, the Linnean Society celebrated the fiftieth anniversary of the announcement of the theory of natural selection in the Darwin-Wallace Essay. Darwin and

Sir Charles Lyell had been dead many years but the other chief actors, Alfred Russel Wallace and Sir Joseph Hooker, were present and spoke to us. And the essential message conveyed by Wallace's speech on that great occasion was a protest against the too great credit which he had been given for his part in the discovery. Of Darwin's part in it he said—"If the persuasion of his friends had prevailed with him, and he had published his theory, after 10 years'—15 years'—or even 18 years' elaboration of it—*I* should have had no part in it whatever, and *he* would have been at once recognised, and should be ever recognised, as the sole and undisputed discoverer and patient investigator of the great law of 'Natural Selection' in all its far-reaching consequences."

It may be fairly claimed that the grip of the profession is less constraining in Applied Biology than in Applied Physics and Chemistry. The notice "No road" is far less likely to be displayed over against some fascinating side-path, because in Biology a side-path so often leads to the goal.

Leaving the application on one side for the moment, it is well to realise the motive which impels to research. When you get down to bedrock you will find that the true inducement is the gratification of the instinct of discovery, indulgence, in fact, in a glorified curiosity. I remember the amusing words in which this conclusion was enforced by Michael Foster. "It was by curiosity" he said "that our first parents lost the Garden of Eden, but by transmitting to us that same curiosity they built for us a golden bridge by which we may re-enter Paradise." And surely the glorious moment when scientific curiosity is satisfied takes us into an earthly Paradise if anything can.

The true motive-power of research is stated by Darwin in a letter to Henslow who had maintained that "However delightful any scientific pursuit may be, yet, if it should be wholly unapplied, it is of no more use than building castles in the air." Darwin in reply pointed to the practical uses which came later but could never have come except with the aid of scientific work conducted solely for its own sake. And he continued: "For myself I would, however, take higher ground, for I believe there exists, and I feel within me, an instinct for truth, or knowledge or discovery, of something of the same nature as the instinct of virtue, and that our having such an instinct is reason enough for scientific researches without any practical results ever ensuing from them."[1]

I do not think that Darwin's argument needs enforcing at the present day, for I believe that everyone will agree with it. You will observe that there is nothing in his words to discourage applied science, provided that its problems are approached in the spirit of which he wrote. And the success which has attended these enquiries is in itself sufficient evidence that they have been undertaken in this spirit. The immense increase in our knowledge of detailed life-histories and the bionomic relations of animals and plants which we owe to the researches in Economic Biology at Washington, in Hawaii, and in this country at Rothamsted and under the guidance of the Imperial Bureau of Entomology and that of Mycology—all this has meant the love of knowledge for its own sake and, without this inspiration, could not have been achieved. If immediate practical benefits result so much the better; if not, the outcome is still as it is in other scientific researches, increase in the mass of human knowledge, out of which practical benefits are certain to come in the end.

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[1] *More Letters*, I, 61.

Any man who has had much to do with scientific students will have realised that many of them—a large proportion happily—are full of scientific curiosity—the love and the longing for discovery—and that they are eager to catch at any suggestion made by one older and more experienced. The suggestions for research in Economic Biology are everywhere around us appealing to the same spirit as that in which the young student begins his first investigation for the love and the glory of discovery alone. If they are not accepted in this spirit they had better not be undertaken at all. But I have no fears. As I have already indicated, the results attained speak for themselves, proclaiming that the work has been done in the spirit of all scientific enquiry, in the intense desire to find out, to know why—the motive cause of all discovery and advance.

Before I conclude there are a few general considerations which I should wish to bring before those of you who are looking forward to a life devoted to the problems of Economic Biology.

The Differences between Island and Continental Areas. The attempt to deal with some economic pest is far less difficult on an island than on the mainland. On the smaller area, with its far simpler web of organic life, there is a good chance of exterminating the evil altogether or of reducing it to comparative harmlessness by the introduction of its natural enemies. Of these some, also attacking other species, may find in the limited fauna little or no food except that provided by the injurious species and will then threaten it, and finally themselves, with extermination. Such a result is far less likely on a continental area, and if it should occur, will only be local and the pest can later on re-invade the locality from the surrounding districts. Again, the simpler environment, inorganic as well as organic, may itself solve or help to solve the problem without any human effort. Storms and other climatic disturbances will tend to produce far greater destruction of life on islands. In a recent attempt to investigate the invasion of the Fijian Islands, and to a less extent other Pacific groups, by certain butterflies and the changes which have taken place in the new home, I have been much struck with the fact that the only evidences of evolution are very modern and superficial and that nothing older has been preserved. There is not only the possibility of hurricanes of exceptional force but of an enemy such as a bird increasing in numbers and entirely destroying some species which it normally only keeps in check. Or again an intruder may itself increase progressively and destroy its own food-plant. All such effects, probable enough on islands, are far less likely to happen over large continental areas.

Unchecked Variation. Whatever the problem before the scientific enquirer he will do well to keep a sharp look out for incidental results of the deepest interest which may often be gained by the way without interrupting the main current of his work or may often-times divert it into a more fruitful channel. The exciting subject of variation is one upon which the Economic Biologist is especially likely to throw light. Under ordinary conditions, with reproduction so far balanced by extermination that the average numbers of a species remain constant, there is reason to believe that variation is held in check by selection. But now and then a time will come—a time in which the help of the Economic Biologist is likely to be required—when a species gets the better of the forces which normally hold it in check, and increases beyond all bounds. The proportionate amount of variation in these temporarily well-nigh selection-free periods is a fascinating subject for study.

The Ebb and Flow of Evolution. The Economic Biologist, like every student of living nature, is sure to consider the bearing of his work upon evolutionary theories and it is therefore well to direct attention to an aspect of evolution which is sometimes neglected. Evolution does not take and probably has never taken a road that leads continuously upward, but one of endless ups and downs, only to be recognised as an advance when averaged over a considerable distance. The upward movement of a single undulation, to be followed at once by an equal or perhaps an even greater descent, is liable to be mistaken for true evolutionary progress. The possibility of such an erroneous interpretation has been forced upon me in studying the changes in the colours and patterns of insects. Colours and patterns were of course evolved from a previous condition of insect life in which they did not exist, and it is tempting to look upon the changes which a pattern undergoes in a series of allied races as part of the great onward movement of evolutionary history. But further investigation shows that the patterns, once formed, have been subject to constant fluctuation, increasing complexity succeeded by increasing simplicity and this again by complexity in endless succession. I do not doubt that the same ebb and flow is true of other aspects of organic nature where it is less easily detected and therefore all the more likely to suggest a fascinating but erroneous interpretation.

THE CROWN-GALL DISEASE OF NURSERY STOCKS

I. FIELD OBSERVATIONS ON APPLE STOCKS

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(With Plates VIII and IX.)

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I. INTRODUCTION.

TUMOUROUS outgrowths in which there are no obvious parasites are of frequent occurrence on cultivated plants and trees, and their cause was for many years a subject for much speculation among growers and plant pathologists. The earlier observers attributed the disease to mechanica injuries or to frosts. Various organisms have also been suggested from time to time as the causal agents, without, however, until comparatively recently, any convincing proof being put forward of their parasitic nature.

About 1890 the galls on fruit trees began to receive great attention in America, where the disease came to be known as "Crown-Gall," because the galls usually arose on the "crown" or "collar" of the trees, at or near the ground level. In 1900 Toumey(12) put forward the view that a myxomycete, *Dendrophagus globosus*, which he found in galls on almond trees, was the cause of the abnormal outgrowths. This theory was not generally accepted and a few years later the subject was re-opened at the United States Bureau of Plant Industry at Washington, where Dr Erwin F. Smith and his colleagues (9) were able to prove by numerous experiments that tumours of the Crown Gall type could readily be reproduced by inoculating plants with pure cultures of an organism isolated from naturally produced galls. This gall-inducing organism they named *Bacterium tumefaciens* Smith and Townsend (8).

Numerous papers have been published by American investigators treating chiefly of various aspects of crown-gall which are out of the scope of the present article. The occurrence of galls on fruit trees has been especially studied by Hedgcock (4, 5), who issued a series of pamphlets on the subject from 1905 to 1911; his Bulletin on "Field Studies of the Crown-Gall and Hairy-Root of the Apple Tree" should be consulted for references to work done on fruit tree galls previous to 1911 and for a description of crown-gall as affecting apple stocks in America. A general account of crown-gall and the experimental work connected with recent investigations will be found in Erwin F. Smith's book (11) *An Introduction to Bacterial Diseases of Plants*. Certain interesting facts relating to galls induced by artificial inoculations, carried out in this country on chrysanthemums and tobacco plants, are discussed in a recent paper by Robinson and Walkden (7).

The interest of the present writers was drawn to crown-gall by reason of the fact that some of the types of apple stocks propagated from layers, which are being raised at East Malling Fruit Research Station, proved to be very susceptible to a disease which apparently was crown-gall, and it was decided to make a study of the disease with special reference to its occurrence on those stocks.

On the Apple Stocks at East Malling it was first noticed about six years ago when it was found on old stools of an unknown variety of "Paradise" stock (referred to here as Type VII), and on trees worked on this stock, but it had previously been observed on the roots of certain ornamental species of *Pyrus* which had been obtained from the Continent. A trace at least of the disease has been found, after close examination, on the subterranean parts of every variety of layered apple stocks so far grown at East Malling, though many of the varieties seem to be moderately resistant when compared with Type VII, which still remains the most susceptible of all.

During the course of the investigation some attention has been given to galls occurring on other hosts and, in addition to those occurring on layered apple stocks, galls, which from their general structure and the absence of any obvious parasites appear to be of the crown-gall type, have been found at East Malling on seedling apple trees, layered pear stocks and layered hawthorn stocks; similar galls on crab stocks, young pear trees, plum trees, rose (particularly Manetti) stocks, raspberry, loganberry, mangel, beetroot, melon, hop and hollyhock have been sent to us from various parts of England.

II. GENERAL OBSERVATIONS ON THE GALLS OF LAYERED APPLE STOCKS.

Our observations on crown-gall have been chiefly directed to the galls occurring on layered apple stocks, either grafted or not grafted, at the end of the second and of the third season after their removal from the parent stools. The method of vegetative propagation called "layering" involves the cutting down of the stock and the earthing up of the resulting shoots to induce them to send out roots; these rooted shoots are severed from the parent "stool" and planted out for nursery purposes (budding, grafting, etc.). It follows therefore that often some six to nine inches of stem (the portion bearing adventitious roots) are planted below ground level.

As a rule the galls found on these stocks grow out from wounds. The wound at the lower end of the shoot, caused when it is removed from the stool, is the most obvious place where internal tissues become exposed, and it is there where many of the galls arise. Such galls, referred to here as "basal galls" (Pl. VIII, figs. 1 and 2), are sometimes almost spherical in general outline, but if the stock has been planted up with a long "heel" an elongated very irregular gall may develop (Pl. VIII, fig. 3). Lateral galls growing out from the side of the stem, from the ground level to the base, here referred to as "lateral galls," are also of frequent occurrence. The basal and lateral stem galls may reach a diameter of several inches in two or three years; their surface is always irregular with numerous rounded swellings from 1 mm. (sometimes less) to 2 or 3 mm. in diameter. Smaller galls often arise on the roots, and on some of the finer roots these have the appearance of rounded nodules which may be isolated (sometimes on the broken or cut end of a root), or several may form a series along the root.

It soon became apparent that the basal galls were larger and more numerous on certain varieties of layered stocks which root from the layer with difficulty and close to the base, and which are consequently often torn from the stool instead of being cut, as are the better rooted layers. Type VII is one of the best examples of this class. Examination indicated that the rough wound made by tearing the stocks from the stools was more liable to infection than a clean cut, and this was borne out by subsequent experimental work (14).

On each of these apple stocks raised from stools, then, there is always at least one wound, and there must often be others which occur during the subsequent handling and planting up. When later these stocks are grafted there is, of course, another wound, but this is usually at some little distance above ground, in the method of grafting adopted in nurseries in this country, and it appears to be exceptional for galls to develop round the junction of stock and scion, as only in a few cases have nodular swellings been found at the grafts.

The relation of this type of basal infection on our layered stocks to the type of infection so commonly reported in America where "bench grafting" is practised, will be obvious. In bench grafting either the whole stock or a piece of the root is grafted during the winter and "heeled in" in a shed or cellar to "callus" until the open ground is in condition to allow of planting. It is then planted rather deeply so that the union between stock and scion is completely covered with soil. It thus results that cut surfaces, not usually fully protected from infection unless special precautions are taken, are covered with sand or soil almost continuously from the time the cuts are made. If the crown-gall organism is present in either the soil or sand, conditions are favourable for its entrance into the young tree. The position of these cut surfaces in the bench grafted trees, just below ground level, gives rise to the frequent occurrence in America of galls at the "*crown*" as opposed to the basal galls of layered stocks in this country.

That the galls may increase considerably in size in the course of a year was shown by the following observations.

During the winter of 1919–20 nine young grafted apple trees were selected, some showing well-developed galls, others irregular callus development at the base suggesting incipient tumours. The parts bearing galls were photographed (natural size where possible) and a record was made of the dimensions of the galls. The trees were then planted up in a small plot at the East Malling Research Station. In the following winter (1920–21) the trees were lifted and the dimensions of the galls again taken; it was then found for example, that a lateral gall had increased from 3·5 (longest diameter) to 5·3 cm., a basal gall from 5·4 to 7·6 cm., and another basal gall from 4·4 to 6·4 cm. (Pl. VIII, figs. 1

and 2). Two of the trees had no distinct galls when planted up, but the callus at the cut end (at base) had wart-like swellings suggesting incipient galls; when examined the following season, however, these were apparently unchanged, so that the warty protuberances were probably merely irregularities in the normal callus growth.

After examination the nine trees were re-planted and again lifted in the winter of 1921–22, when the galls were compared with previous records. It was found, however, that on this occasion no further increase whatever in the size of the galls could be detected, except in the case of a small gall on a root. The trees themselves had made but little growth during the previous summer[1] and it was probably this that had had an adverse effect on the development of the galls.

When examined in the winter 1922–23, there was no further increase though the galls still showed no signs of decay, and the experiment was discontinued.

The galls found on the two, three and even four-year-old apple trees examined at East Malling, showed no signs of disintegration, but on a young apple tree received from another nursery in Kent there was a gall which was brown in parts and evidently in an advanced stage of decay. It is generally supposed that as the galls become disintegrated they liberate the gall-producing organisms into the soil which thus becomes further infested. Robinson and Walkden (7) find that in the case of chrysanthemum galls "there are enormous numbers of bacteria situated on the exterior of the gall, that a high percentage of these is *B. tumefaciens*, and that it is reasonable to assume that the presence of these progressively increasing numbers of *B. tumefaciens* on the exterior provide the progressive stimulus which leads to the continued growth of the gall." If it should prove that a similar condition obtains in such underground galls as those under consideration here, it would seem that the soil would become automatically infested in the neighbourhood of the galls while they are still intact and developing.

The consistency of these apple galls and their continued growth from year to year indicate that they are of the type known as "hard galls" by American investigators. "Soft galls" have not been seen on the layered apple stocks at East Malling. On one occasion, however, a two-year-old seedling with a well-developed hard gall was planted up in a pot, and when examined the following year, it was found that the hard gall had increased in size and was quite sound while near it there had developed a soft gall (about an inch in diameter) which was brown in colour and swarming with eelworms; whether the eelworms or bacteria were the primary cause of the soft gall was not determined.

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[1] This result was doubtless due to (1) the abnormally dry summer of 1921, and (2) the fact that trees of that age may be greatly checked by frequent transplanting.

Only one instance of what appears to be a true case of "hairy-root" (a condition commonly associated with crown-gall in America) has come under our notice on the East Malling stocks. A young apple stock, with several clusters of fibrous roots, bore at one node a tuft of roots which were finer and softer to the touch than the rest. An organism has been isolated from the swollen base from which these roots arose and is being compared with others isolated from the usual type of apple gall. The very plentiful development of fibrous roots on some of our apple stocks appears to be typical of certain varieties and, so far as we can see, has no connection with crown-gall.

III. RELATIVE SUSCEPTIBILITY OF THE DIFFERENT STOCKS.

Observations extended over four years tend to show that the different varieties of layered stocks differ widely in their susceptibility to crown-gall. Some varieties show year after year not only a higher proportion of galled stocks or trees but also larger galls than others. Although the order of stocks in regard to the amount of infection observed varies in detail from one season to another, two varieties, Type VII and a layered "free" stock known to us as "No. 51," stand out as being always the most highly susceptible of all.

Those stocks of which a considerable quantity (mostly in the form of one year and two year old trees) have been available for examination may be roughly divided into three groups. In the first group, which may be called "very susceptible," come the two varieties mentioned above, Type VII and No. 51. Under the conditions in which they were grown 88 per cent. of one year old and two year old trees on these stocks showed at least a trace of crown-gall, nearly 50 per cent. of the trees could be classed as conspicuously galled, and of these 25 per cent. actually had galls one inch or more in diameter. The second group may be called "moderately susceptible"; of these the most susceptible is undoubtedly Type II (Doucin), which at times has shown nearly 80 per cent. of trees with at least a trace of crown-gall, but here the proportion of conspicuous galls is very much lower and rarely exceeds 15 per cent. of the whole, even in the case of Doucin, whilst others of the group show still less infection. The third group, which may be called "moderately resistant," includes several varieties which rarely (under our conditions) show as many as 50 per cent. of trees with traces of galls, whilst galls one inch or more in diameter are often entirely absent and seldom exceed 2 or 3 per cent.; in this group come Type I (the "Broadleaf" Paradise), and Type VI ("Nonsuch" Paradise).

It has been already mentioned that those varieties of apple stocks which root with difficulty when propagated vegetatively show a relatively high degree of susceptibility, and this is emphasized by the grouping. Both of those in the first group are rooted only with difficulty; the same is true of the Doucin, Type II, the most susceptible of the second group. The most resistant of the third group, Types I and VI (Broadleaf and Nonsuch), are amongst the most easily rooted of all apple stocks. But this difference in rooting, and the necessity for tearing the shy rooting stocks from the stools, will not account for all the differences in susceptibility. Type VII, for instance, is much more easily rooted than No. 51, and somewhat more easily than Type II, yet it has invariably been the most susceptible. It may safely be said that there are real, inherent differences in susceptibility amongst the varieties, besides those apparent differences which arise as a result of the particular mode of removal from the stools.

A point of some interest is that there is no close correlation, so far as our observations go, between the relative "vigour" of a variety and its degree of susceptibility. Each of the three groups includes varieties widely different in their vigour; of the two most vigorous stocks, one, No. 51, is "very susceptible," whilst the other, Type XII, is "moderately resistant"; the "moderately susceptible" group includes several of the very vigorous Continental varieties of Paradise stocks, together with Type IX (the Jaune de Metz), one of the weakest growing apple stocks known. It would seem then that there are factors other than vigour that determine the degree of susceptibility of any particular variety.

This conclusion does not imply that there is no correlation between the size of the galls and the vigour of the individual trees bearing them, *within the variety*; in fact, from analogy with results obtained from experimental work on other species of plants it would appear probable that, in the case of fruit trees also, the rate at which a gall develops depends on the vigour of the host as influenced by soil conditions and other environmental factors. Levine (6) has shown that in the case of mangels infected with crown-gall the vigorous plants (grown in rich soil) produced large galls while the weak plants (grown in poor soil) gave rise to small galls.

IV. SUSCEPTIBILITY OF THE STOCKS AS INFLUENCED BY THE SCIONS.

In the observations described in the preceding section the possible influence of the scion with which a tree is "worked" is eliminated, since the results were all based on equal numbers of stocks grafted with the two varieties Bramley's Seedling and Worcester Pearmain. As our observations proceeded evidence was obtained that this factor, the influence that the scions may have on the number and size of the galls on the stocks, must be taken into consideration in determining the relative susceptibility of different stocks. Thus, in the case of three varieties used as scions in 1919, it was subsequently found that the galls on the stocks worked with either Lane's Prince Albert or Worcester Pearmain showed a much greater development than did those on the stocks worked with Bramley's Seedling. This point has been followed up, and large numbers of trees of many varieties have been examined each winter.

Unfortunately it has not been possible since 1919–20 to examine very many trees of any variety on a highly susceptible stock; we were thus obliged to rely for trustworthy figures on stocks which are somewhat less susceptible, such as Type II. Even here, however, the figures repeatedly show a potent influence of the scion on the development of the galls.

Of all the varieties examined, Lord Derby appears usually to render the stocks more susceptible to crown-gall than any other, followed by Early Victoria and Lane's Prince Albert. On the other hand, Bramley's Seedling, Bismarck, Newton Wonder and Stirling Castle appear to render the stocks more resistant than most other varieties.

The influence of the Lord Derby scions on stocks is correlated with a high degree of susceptibility in the variety itself, as was recently seen when some half dozen trees of this variety on their own roots (grown from layers) were lifted for transplanting. Nearly all had large galls, and none could be regarded as less than moderately galled. For so few trees they were undoubtedly one of the worst batches ever lifted at the East Malling Station. It seems likely that the Lord Derby variety (on its own roots) will prove to be even more susceptible to crown-gall than Type VII.

V. OBSERVATIONS ON GALLED TREES IN THE PLANTATION.

An exact knowledge as to the effect that crown-gall has on fruit trees in this country is wanting, and the evidence supplied by horticulturists in America is somewhat contradictory. In some cases, however, careful records have been made by American workers on a large number of trees, some healthy and others with galls, and the results obtained go in the direction of showing that the galls have an adverse effect. One of the most recent papers on the subject is by Greene and Melhus (3), who obtained records of the rate of growth and amount of fresh growth made from year to year by young apple trees; the chief points in their summary of results are:

"In this experiment galled trees did not grow nearly as rapidly as normal trees. Those which were galled when set and later recovered made a greater growth than those that did not recover from the galls.

"The data presented show that crown-gall greatly retards the growth activities of young apple trees. It is followed by a reduction in the amount of increase in trunk diameter, and in the number of twigs, their length, their thickness and their weight."

An experiment on somewhat similar lines has been carried out at East Malling with, however, inconclusive results. Records have been made for a considerable number of trees of Bramley's Seedling and Worcester Pearmain worked on Types II and VII, some of which bore galls (the size of the gall on each galled tree was carefully noted before planting), the others being free from galls at the time the trees were planted up. In the average length of new wood formed each year, and also in the average number of fruit buds, the variation seems to be entirely independent of the presence or absence of galls at planting, and of their size and position on the stock. It was thought likely that lateral galls (on the stem, just below ground level) might have a more adverse effect than basal galls, yet the records obtained do not indicate any such difference. In one case, five trees of Bramley's Seedling on Type VII, noted at planting as having large lateral galls, actually made on the average from 25 to 30 per cent. more growth, as estimated from the length of young shoots produced, in both 1920 and 1921 than a similar group of four trees noted as being only slightly galled at the base. In other cases the difference is reversed. On the average of a large number of trees, those noted as being more or less galled when planted have made at least as much growth, and have blossomed to approximately the same extent, as those noted as "clean."

It would appear then that, under the conditions which obtain at East Malling, the galls produce at any rate no striking effect on the general health of the trees after they are planted up in their permanent quarters. The records however do not include any account of the possible effect of the galls on the young trees previous to their being planted up. As mentioned below some 40 per cent. of the total weight of a young seedling apple tree may be gall tissue. In some Manetti rose stocks the galls have been found to be 50 per cent., or even more, of the total weight. It would appear inconceivable, in such cases, that the galls are not in some way detrimental, if only because of their absorbing food which should have been utilised in developing the normal organs, and the fact that microscopic examination shows that the cells of apple galls contain large quantities of starch is not without significance. Fracker's (1, 2) observations on apple nursery stocks are of interest in this connection; in an examination of 1012 young apple trees he found that 65·1 per cent. of the non-infected trees could be classed as Grade I, while only 37·7 per cent. of trees bearing galls or infected with hairy root were sufficiently large and strong for that grade.

VI. OBSERVATIONS ON GALLS FOUND ON SEEDLING APPLE TREES.

From our earlier observations, *i.e.* on layered stocks, there was no good evidence that the galls originated anywhere except at the wounds produced during the process of removing the stocks from the stools and planting them up, and (rarely) at the grafts. More recently however it was found that well-developed galls may occur on seedling trees in the absence of obvious wounds. Thus a crab stock was found to have numerous galls on its roots, the largest of these galls being over an inch in diameter, and there was nothing to show that the roots had been injured previous to the development of the galls (Pl. IX, fig. 4). Again, a number of two-year-old seedlings raised at East Malling from pips of cider apples have been found with comparatively very large galls (Pl. IX, figs. 5 and 6), although the trees had not been moved, since the seeds were sown, until they were taken up at the end of the second year; the galls were mostly at the "crown" of the trees, which appears therefore to be a particularly susceptible point in the case of apple seedlings. Compared with the size of the trees some of these galls were of considerable size; thus in one case a young tree with one gall was found to weigh 17·9 gm.; the gall was then cut off and its weight ascertained to be 7·8 gm. or 43·6 per cent. of the total weight of the tree. Of some 2000 seedling trees examined, barely 100 (or about 5 per cent.) were entirely free from galls —a very small proportion considering the conditions under which the trees were raised.

How infection arose in the case of such seedlings is at present not clear. Possibly the galls arose in some instances from wounds produced during cultivation; injuries caused by wireworms and other insect larvae are also suggested as providing an entrance for the gall organism. E. F. Smith (10) has shown that in certain experiments, where artificial inoculations were made in the region of rapidly growing tissues, "secondary galls" arise at some distance from the wound where the "primary gall" develops. Although his interpretation of their mode of origin is criticised by Robinson and Walkden (7) they confirm his observations on the fact that the secondary galls arise from within and in the absence of any external injury at those particular points. Whether galls arise in a similar manner in the more woody tissues of apple roots is not known, but the way in which the galls sometimes arise in series along the roots on seedling trees, and occasionally also on layered stocks, suggests that perhaps some of them arise as secondary galls.

VII. AIM AND SCOPE OF WORK IN PROGRESS.

The field observations described in the preceding pages have suggested lines for further investigation, and research is in progress to obtain information on the following points.

(1) *The Cause of the Galls.* That the galls occurring on apple stocks in Britain are a result of the invasion of the tissues by a micro-organism is suggested by their close resemblance to the galls found on nursery stocks in America where investigation has shown them to be due to the presence of *Bacterium tumefaciens*. Bacteria, which, in preliminary cultural tests[1], show affinity with *B. tumefaciens*, have been isolated from apple galls found on layered apple stocks at East Malling, and inoculation experiments carried out with pure cultures on young apple stocks have yielded, in certain cases, tumourous outgrowths; these positive results, however, up to the present, are too few in number for definite conclusions to be drawn as to the parasitic nature of the organisms isolated, and further inoculation experiments are being carried out. That *B. tumefaciens* does occur in this country was announced in 1921 by Walkden (13), who isolated it from galls found on *Chrysanthemum frutescens*.

As already mentioned, tumours of the crown-gall type have been found on a number of species of cultivated plants in this country. Whether all such galls are caused by micro-organisms and whether the parasite, if present, is, in each case, identical with the American species, *B. tumefaciens*, are points which require elucidation.

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[1] The preliminary cultural and inoculation experiments were carried out at the South-Eastern Agricultural College, Wye, and the work is being continued at the East Malling Research Station.

(2) *Effect of Galls on the Vigour of Nursery Stock.* It has been shown that no essential difference has up to the present been detected at East Malling in the general vigour of trees subsequent to their being planted up with or without galls. Further work is required, however, particularly as to the effect the galls may have on the young ungrafted trees, and also on the grafted trees while they are still in the nursery. Reference has been made to Fracker's observations, but the results need to be confirmed by direct experiment using uniform stocks. Material for such an experiment is available at East Malling, and when a method of inducing gall development as required has been found, a comparison will be made of infected and uninfected young stocks.

(3) *Control Measures.* The fact that, in the case of layered apple stocks, the galls arise chiefly at wounds suggested that a possible method of control lay in reducing to a minimum the surfaces liable to infection. Field experiments with this object in view were initiated in 1920, and from the results of experiments already carried out(14) it would appear that the number and the size of the galls may be reduced by avoiding unnecessary injuries, and by covering the wounds (made when removing the stocks from the parent stools) with a protective layer of Stockholm tar or grafting wax. A more detailed account will be published when the results of a further series of experiments, now in progress, are to hand.

Again, since infection occurs on planting the stocks in infected soil, it appears probable that the crown-gall organism is capable of living saprophytically in the soil, so that another possible means of control is in a suitable treatment of the soil, either by judicious manuring, or by the application of a bactericidal substance. Whether treatment along these lines will prove practicable remains to be proved, but biological studies of the apple crown-gall organism may indicate the best way of approaching the problem.

(4) *Identification of Incipient Galls.* Another feature of interest to the plant pathologist is the diagnosis of the disease in its early stages. The irregular callus that develops over a ragged wound may resemble an incipient gall, and such irregular callus development is frequent on nursery stocks which are propagated vegetatively, especially if the shoots are torn away from the stools. At what stage it is possible to distinguish between a young gall and normal callus has not yet been determined, but when a successful method of inducing galls on apple stocks by artificial inoculations has been found a comparison of young galls and the uninfected callus of control plants will be made.

SUMMARY.

An account is given of the galls ("crown-gall") found on layered apple stocks and on seedling apple trees raised at the East Malling Fruit Research Station.

On the layered stocks the galls arise most frequently at the base of the stocks, *i.e.* at the wound caused when removing the stock from the parent stool, but they may also occur on other parts of the stem below ground, and on the roots.

Observations extending over several years lead to the conclusion that, although galls have been found on every variety of layered apple stocks so far propagated at East Malling, some varieties are far more susceptible to crown-gall than others.

Evidence has been obtained that the susceptibility of the stock is to some extent influenced by the variety of scion grafted on it.

No difference in vigour could be detected on the subsequent general growth of trees planted up with, or without, galls.

BIBLIOGRAPHY.

(1) Fracker, S. B. Is crown-gall injurious to apple nursery stock? *Journ. Econ. Entom.* xi (Feb. 1918), 133–5.

(2) —— Effect of crown-gall on apple nursery stock. *Phytopath.* viii, No. 5 (May, 1918), 247.

(3) Greene, L. and Melhus, I. E. The effect of crown gall upon a young apple orchard. *Res. Bull,* No. 50, *Agric. Exp. Station, Iowa Coll. Agric.* (Mar. 1919), 147–76.

(4) Hedgcock, G. G. The crown-gall and hairy-root diseases of the apple tree. *Bull.* 90, pt. 2, *Bur. Plant Ind. U.S. Dept. Agric.* (Nov. 1905), 5–7.

(5) —— Field studies of the crown-gall and hairy-root of the apple tree. *Bull.* 186, *Bur. Plant Ind. U.S. Dept. Agric.* (Nov. 1910), 108 pp.

(6) Levine, M. Studies on Plant Cancers. III. The nature of the soil as a determining factor in the health of *Beta vulgaris* and its relation to the size and weight of the Crown Gall produced by inoculation with *Bacterium tumefaciens. Amer. Journ. Bot.* viii, No. 10 (Dec. 1921), 507–25.

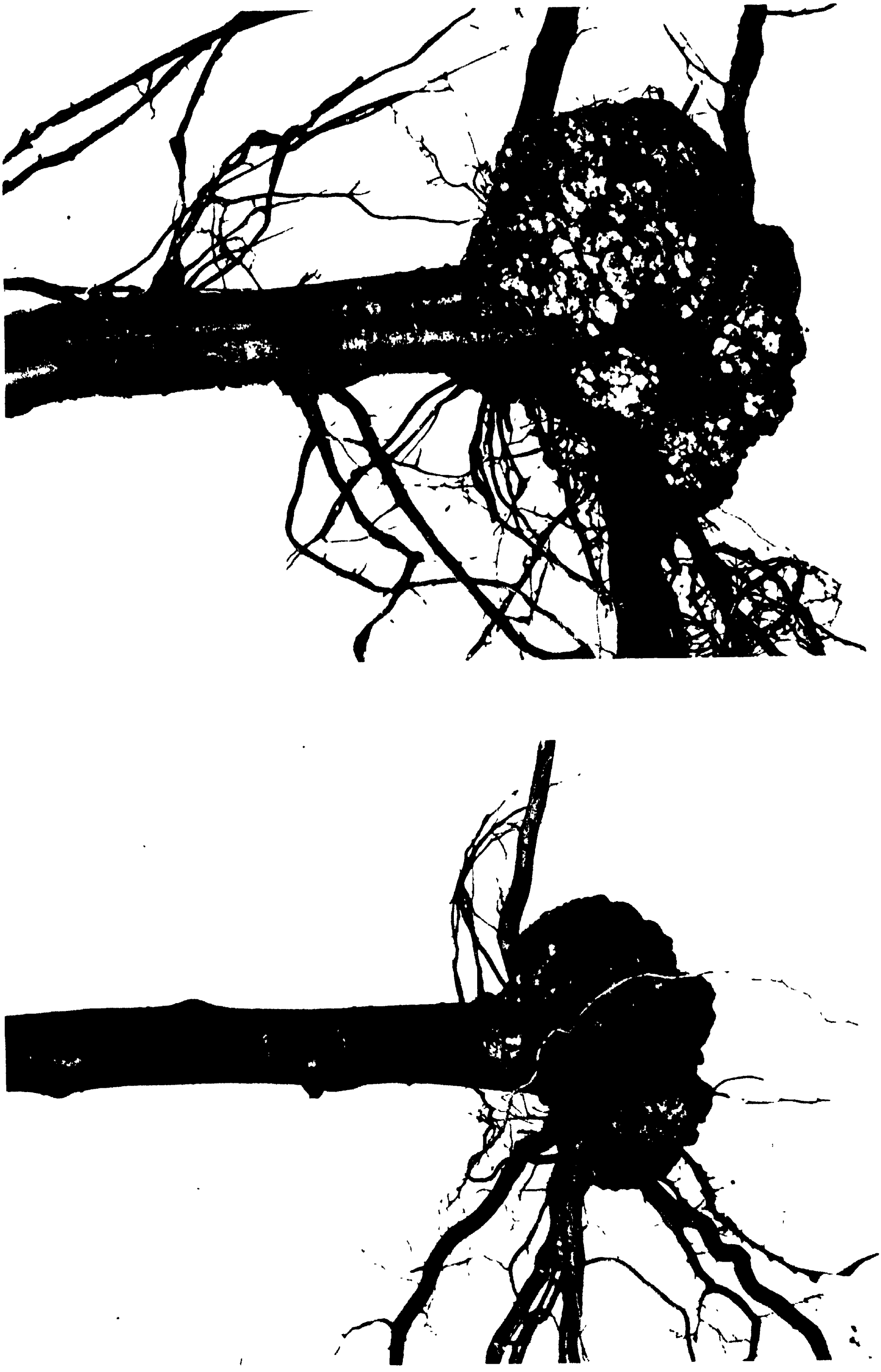
(7) Robinson, W. and Walkden, H. A critical study of crown-gall. *Ann. Bot.* xxxvii, No. 146 (Apr. 1923), 299–324.

(8) Smith, E. F. and Townsend, C. O. A plant tumor of bacterial origin. *Science,* N.S. xxv (Apr. 1907), 671–3.

(9) Smith, E. F., Brown, N. A. and Townsend, C. O. Crown-gall of Plants: its cause and remedy. *Bull.* 213, *Bur. Plant Ind. U.S. Dept. Agric.* (Feb. 1911), 215 pp. and 36 plates.

(10) Smith, E. F., Brown, N. A. and McCulloch, L. The structure and development of crown-gall. *Bull.* 255, *Bur. Plant Ind. U.S. Dept. Agric.* (June, 1912), 60 pp. and 109 plates.

(11) Smith, E. F. *An Introduction to Bacterial Diseases of Plants.* Philadelphia and London. 1920.



WORMALD & GRUBB.—The Crown-Gall Disease of Nursery Stocks. (pp. 278—291.)

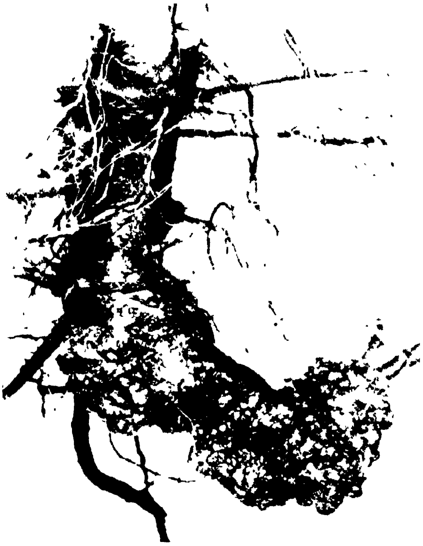


Fig. 3.

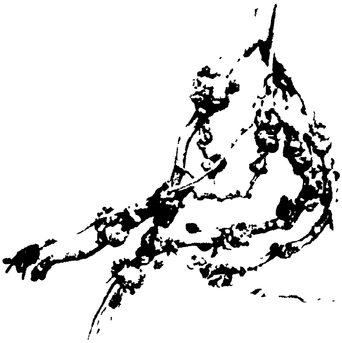


Fig. 4

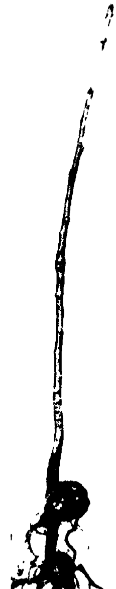


Fig. 5.

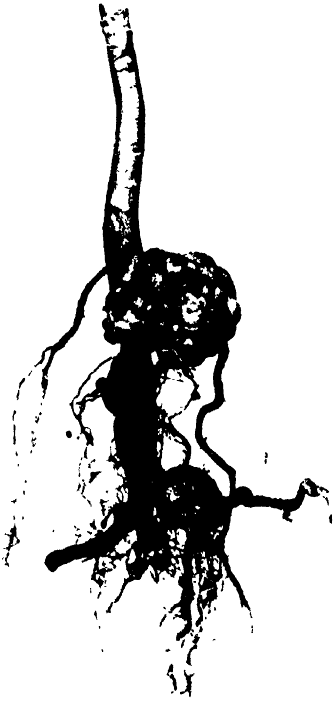


Fig. 6.

WORMALD & GRUBB.—The Crown-Gall Disease of Nursery Stocks. (pp. 278—291.)

(12) Toumey, J. W. An enquiry into the cause and the nature of crown-gall. *Bull.* 33, *Arizona Agric. Exp. Station* (1900), 29–30.

(13) Walkden, H. The isolation of the organism causing crown-gall on *Chrysanthemum frutescens* in Britain. *Ann. Bot.* xxxv, No. 137 (Jan. 1921), 137–8.

(14) Wormald, H. and Grubb, N. H. Notes on the control of nursery stock against crown-gall. *Gard. Chron.* lxxi, No. 1843 (Apr. 1922), 198.

EXPLANATION OF PLATES VIII AND IX.

PLATE VIII.

Fig. 1. A typical basal gall on a layered apple stock. (Nat. size.)

Fig. 2. The same a year later. (Nat. size.)

PLATE IX.

Fig. 3. A stock torn with a long "heel" from the stool; galls are developing all along the wound. × $\frac{3}{8}$.

Fig. 4. The root-system of a crab stock badly galled. × $\frac{1}{4}$.

Fig. 5. A two-year-old apple seedling tree with a gall at the collar, showing relative size of tree and gall.

Fig. 6. The gall (seen in Fig. 5). × $\frac{5}{7}$.

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SOME OBSERVATIONS ON THE GROWTH OF *RHIZOCTONIA CROCORUM* (PERS.) DC. IN PURE CULTURE

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(With Plate X and 3 Text-figures.)

Name of the fungus.

The fungus with which these notes are concerned is that which causes the well-known "Violet Root Rot" of various crops, such as lucerne, potatoes, carrots, etc., and which has hitherto been generally known in this country as *Rhizoctonia violacea* Tul. The specific name *violacea* was given by the Tulasne brothers (1) in 1851 when they reduced to a single species the older forms known as *R. Crocorum* and *R. Medicaginis*. This proceeding is however contrary to the Rules of Nomenclature now accepted. Assuming, as do the majority of workers, that the Tulasne brothers were right in their view that the violet root disease in all the various crops is caused by one and the same species of fungus, even though there may be biologic forms, then the earliest valid name, and not that of the Tulasnes, must be adopted. As pointed out by Duggar (2) the correct combination is *Rhizoctonia Crocorum* (Pers.) DC. The specific name was originally used as *Sclerotium Crocorum* by Persoon (Synopsis, 1801) for the form on *Crocus sativus*. It was transferred to his genus *Rhizoctonia* by De Candolle in 1815, and both this and De Candolle's second species, *R. Medicaginis*, were taken up by Fries in the Systema Mycologicum (1823), which latter is the starting point for Nomenclature according to the International Rules. It is unfortunate that the name is less descriptive than *R. violacea*, and is particularly inappropriate in this country where the Crocus disease is unknown. Pending further information as to the perfect stage, however, the name *R. Crocorum* must stand.

Origin of the present work.

In the winter of 1922–23 a considerable amount of circumstantial evidence was obtained pointing to the possibility that *R. Crocorum* might have as its perfect stage the fungus *Helicobasidium purpureum*. The authors therefore set out on an attempt to test the theory by pure culture and inoculation experiments. They were fortunate in being able to obtain comparatively abundant material of the *Rhizoctonia* on clover and later also of *Helicobasidium* through the kindness of Mr W. M. Ware of the South-Eastern Agricultural College, Wye. Owing to unexpected complications it has not yet been possible to obtain sufficient evidence for a definite pronouncement, but the authors hope to be able to give some account of this investigation after a more complete set of inoculation experiments has been carried out.

The present notes are the outcome of one side of the work, namely, the successful and continued growth of *R. Crocorum* in pure culture. The only previous records of artificial cultures of this fungus known to the authors are those of Diehl (3) in America and van der Lek (4) in Holland, neither of whom carried out very extensive cultural work with it. The former grew the *Rhizoctonia* chiefly in mixed culture with a species of *Fusarium* and did not obtain good growth in the pure state. Van der Lek succeeded in isolating the fungus pure from sections of the "infection cushions" or "corps miliaires" and obtained good growth on malt agar. He also grew it on cherry and dung extract agars and gives some account of variations in colour according to the medium and the amount of moisture present. We understand that both of these workers allowed their cultures to die out. Faris (5) writing in 1921 stated that "cultural experiments to secure pure cultures of the organism (*R. Crocorum*) are under way" but the authors have seen no account of successful cultures. In a letter to one of the authors Dr G. H. Pethybridge stated that he grew the fungus successfully, although not in pure culture, by keeping an attacked potato tuber in a covered dish with other healthy potatoes. In time the *R. Crocorum* grew luxuriantly over the whole lot and its "corps miliaires" penetrated the skins.

METHODS USED FOR ISOLATION IN PURE CULTURE.

After numerous unsuccessful attempts in the winter of 1922–23 growth of *R. Crocorum* was obtained in pure culture on malt agar and on a meat and malt extract gelatine acidified with citric acid[1] by adopting the method of van der Lek (4), viz. planting moderately thick sections of "infection cushions" of the fungus, cut with a razor under aseptic conditions, on previously poured plates of the media. One of the sections showing promising growth was sacrificed for high power microscopic examination and the developing hyphae were seen to be growing out from the centre of the micro-sclerotium as described by van der Lek. The material used was that on Red Clover, mentioned above, supplied by Mr W. M. Ware (cf. (6))[1]. Van der Lek (*loc. cit.*) gives no details of his method of surface sterilisation of his plant material. The authors obtained best results from the use of a 50 per cent. alcoholic 1/1000 mercuric chloride solution with technique similar to that adopted by bacteriologists (cf. Manns (7)). Portions of the clover root bearing numerous infection cushions or micro-sclerotia were put into sterile test tubes and well covered with the mercuric chloride solution. After a given interval the disinfectant was poured off and the material washed at least three times with separate portions of sterile distilled water. Each time the liquid was shaken to all parts of the tube and on changing the liquid the old cotton wool plug was replaced by the dry sterile plug from the new sterile water tube. The material remained in the original tube throughout the operation. It was found that about one minute in the mercuric chloride solution was ample to kill all really surface contaminations: longer exposure usually resulted in absolutely sterile sections—killing also the micro-sclerotia of the *Rhizoctonia*. After surface sterilisation the material was transferred to a sterile petri dish, portions cut off from the root tangentially ½–1 mm. thick and transverse sections of the micro-sclerotia cut in sterile pith. The razor was flamed after dipping in alcohol before use. The difficulty which has been experienced by previous workers (cf. Duggar, *loc. cit.*) in obtaining growth of *R. Crocorum* on artificial media has been occasioned in part by the apparently low degree of vitality possessed by such minute portions of mycelium as can be transferred without accompanying contaminations from the advancing edge of growth of the fungus, *e.g.* at the collar of infected plants. In the opinion of the authors the main difficulty has been due *not* to the fungus being on the borders of obligate parasitism but to the difficulty of seeing and isolating the hyphae, which grow out slowly from viable portions of the fungus, *e.g.* the micro-sclerotia, before they have been swamped by other more quickly developing fungi and bacteria. In the field it is rare to find many plants attacked by the parasite and the choice of material is rather limited. Further, from the fact of the *Rhizoctonia* occurring mainly on the underground parts of plants, it is always associated with very numerous rapidly growing saprophytes and in many cases before the attacked parts are dug up these saprophytes have already obtained a hold on the tissues affected by the *Rhizoctonia*. In cases where the razor sections of the micro-sclerotia were "planted" free from contaminations and where the surface sterilisation of the roots had not been too severe the *Rhizoctonia* grew out slowly but steadily on to the artificial medium[1]. When suitable conditions were found for the growth of the fungus it grew at least as freely in culture as some other parasitic fungi whose occurrence on the aerial parts of plants comparatively free from contaminations, combined with the production of abundant conidia, has led to their being isolated in pure culture without difficulty. The very slow growth of certain parasites, such as species of *Pseudopeziza*, in artificial culture has been noted particularly by Klebahn (8).

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[1] This gelatine was made up with tap water 1000 c.c., malt extract 20 gm., Lemco meat extract 10 gm., citric acid 4 gm., gelatine 120 gm., and cleared with white of egg.

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[1] A further case in this country of a very slight attack of *R. Crocorum* on Red Clover has since been noted by us in Hampshire. *R. Crocorum* has also occurred in one of our experimental pots on another species of clover (*Trifolium hybridum*).

CONFIRMATION OF THE IDENTITY OF THE ORGANISM ISOLATED.

The mycelium which grows out from the infection cushion or micro-sclerotium is at first colourless, and the hyphae very slender, but very soon the characteristic features of the branching of *Rhizoctonia* can be detected. After the lapse of two to three weeks at laboratory temperature the colony shows the typical violet colour and the older hyphae are now seen to be assuming the size and colour characteristic for *R. Crocorum*. The centre of the colony gradually becomes deep violet or brownish, according to the medium, and at the same time the substratum becomes permeated with the densely matted branching hyphae, so that a tough, compact mass is formed which is very difficult to cut with the usual platinum needles used for making sub-cultures.

The hyphae of *R. Crocorum* obtained in culture do not differ essentially from those of the fungus in nature, as described and figured by Duggar (*loc. cit.* p. 415). The normal well-grown hyphae are usually about 4–6 μ in diameter. The younger hyphae are pale, especially towards the tips, with rather distant transverse septa. At the apices the hyphae contain dense protoplasm, but further back they become vacuolate, while older hyphae become thick-walled, rigid and deeply coloured, and appear to contain little or no protoplasm. Branches arise usually singly, more or less at right angles to the main hypha, and near to the cross walls. Each branch is cut off by a septum close to the main hypha, and frequently appears to be slightly narrower near the point of origin. Duggar's observation that at the point of union the diameter of the branch is uniform with that of the main hypha applies more to the older, coloured hyphae. The experience of the writers has been that the younger hyphae, both in nature and in culture, usually show this slight narrowing where the branches join the parent hyphae. The same observation has been made by Faris (5). Occasional fusions between adjoining hyphae take place, but no clamp connections have ever been observed.

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[1] A contributing factor to the slow growth obtained at first was that it was necessary to use a fairly thin layer of medium, with consequent drying out, in order to be able to watch the growth with the low power of the microscope through the bottom of the petri dish.

In order to confirm the results of microscopic examination and to show more definitely that the fungus isolated was really *R. Crocorum* inoculation experiments were carried out. As a preliminary test, on 29th July, 1923, portions of mycelium from a pure culture were placed in contact with young carrots and potatoes, the surface of which had been carefully washed, treated with dilute formalin and again washed. Two potatoes and three young carrots thus inoculated were kept moist in a large covered glass dish. In the case of the potatoes there was a very slow growth of scattered hyphae over the surface of the tubers but no further development. With the carrots, however, better results were obtained. On 1st September one of the carrots showed a distinct patch about 1 cm. in diameter of typical *R. Crocorum* attack with minute infection cushions, while a second root showed also incipient attack and a few infection cushions. In both cases the affected parts became gradually much larger until finally both carrots were completely destroyed. Secondary organisms no doubt contributed to the rot.

A further inoculation experiment was carried out with young growing carrots. Some seedling carrots had been carefully transplanted into two large pots of soil which had been sterilised by steam by the usual method for gardening operations. By 30th July these were well established and sufficiently large for inoculation. In one pot fairly big pieces of growth cut from pure culture were inserted in the soil close to the crown of each plant. The second pot was left untouched as a control. By 17th September one carrot in the inoculated pot was seen to be dying off and when pulled up was found to be covered with the typical infection cushions of *R. Crocorum.* By 18th October a second carrot had completely rotted and the skin remaining was found to be full of infection cushions. Later a third carrot died and was found to be attacked in the same way, while another showed slight infection. In the control pot no sign of *Rhizoctonia* ever occurred. From one of the infected carrots the parasite was re-isolated by the same method as used originally. The growth in artificial culture was found to be exactly similar to that obtained from the clover material.

A similar inoculation test was carried out with Red Clover. Seeds of Red Clover had been sown in large pots in sterilised soil in July 1923 and on 3rd September the young plants in one pot were inoculated by placing portions of a pure culture in the soil close up against the upper part of the tap-root of each plant. On 2nd November further mycelium from a pure culture was introduced. A pot was left uninoculated as a control. The pots were kept in a small unheated glasshouse, and as from the time of the first inoculation the weather was never warm results were doubtless obtained much more slowly than they would have been had it been possible to inoculate earlier in the season. On 11th April 1924 all the clover plants were pulled up and examined. Of those in the inoculated pot five out of eight showed typical attack by "Violet Root Rot." Infection cushions occurred both on the tap-roots and on some lateral roots, while in one case the tap-root had completely rotted away leaving only a short stump. The clovers in the control pot were found to be all quite healthy.

There seems to be no doubt therefore that the organism isolated and grown in pure culture is really *R. Crocorum.*

The successful inoculation of carrots with a pure culture obtained from clover plants is of some significance in connection with the view expressed by Eriksson(9) that the violet root rot of the carrot and of lucerne (a legume) are two entirely different things. Eriksson considered that the sterile fungus called *R. violacea* (*R. Crocorum*) might include several biologic forms or even species. He based this opinion in the first place on the results of some cultural experiments. Using infected soil in which a crop of diseased carrots had been grown, he got only a slight infection on lucerne and none on Red Clover or parsnip; in one experiment made with cross-inoculation in the reverse direction, from lucerne to carrot, he got no result. Similarly a test with forms of the fungus on lucerne and asparagus indicated that these would not pass from one host to the other. From these experiments and from records of the occurrence of the alfalfa (lucerne) disease and of attacks on Red Clover in Germany (the former frequent, the latter rare) he argued even that the lucerne and the clover forms might be distinct.

Seeing that our present knowledge is so slight, however, both as to the requirements of the fungus and the conditions necessary for successful infection of its various hosts, Eriksson's experiments require to be carefully repeated.

Eriksson's further argument that the perfect stage of the carrot fungus is his *Hypochnus violaceus* and that of the alfalfa fungus *Leptosphaeria circinans* is generally regarded as being based on insufficient evidence, and need not be considered here.

GROWTH ON VARIOUS MEDIA.

Diehl (3) obtained his best growth of *R. Crocorum* on "radicicola" agar acidified to 5 on the scale given in old editions of the American Standard Methods of Water Analysis. As the authors were unable to have access to an old edition it was impossible to find how much added acetic acid was represented by the number 5, but from the description given by Diehl it would appear that very much better growth was obtained on the media generally used in the present work than that given by the acidified "radicicola" agar. Van der Lek obtained good growth on malt agar.

The authors have found that media made up with malt or meat extract or preferably both, and also prune agar, allow of best growth, and these have been chiefly used. When first isolated, however, transfers were made to a great variety of media with the aim of finding as early as possible which would be the most suitable, so that there should be no risk of the organism dying out when once obtained in pure culture. Pure growth was sparse at the time so that only a few tubes of each were inoculated. Further, in some cases the agars available had been prepared for some time, and it has been noted from later work that there may be appreciable differences, particularly in the amount of aerial growth, when freshly tubed agars are used. The results are, however, given here in brief in order to show on how wide a range of sterilised artificial material growth of *R. Crocorum* will take place when it is not overwhelmed by the presence of other more rapidly growing organisms.

As an illustration of the way in which the development of aerial mycelium, and hence the general appearance of the culture, depends on the amount of condensation moisture present one case may be given where four malt-meat extract agar plates were poured at the same time, and inoculated with as nearly as possible equal and very minute fragments of mycelium from the edge of a recent culture. The plates were kept in an incubator at 22–23° C. It happened that two of these plates included a considerable amount of water of condensation while the other two remained fairly clear and dry. After about four weeks the differences in growth were striking. In the two plates with much moisture there was good development and a soft growth of pale lilac-brown aerial hyphae similar to that occurring in fresh tubes. The plate with most free moisture gave a colony 25 mm. in diameter while the one with slightly less moisture measured 13 mm. The other two plates, which had remained clear and dry, showed very much less growth—less than 10 mm. in diameter—and the mycelium was practically entirely submerged, the colony therefore appearing dark purple in colour with no paler aerial growth. On the other hand, while endeavouring to obtain conditions under which the fungus would give regular and equal growth on duplicate plates in artificial culture it was grown on malt extract and meat-malt extract agars made up with 1, 1½, 2, 3 and 5 per cent. of agar, and the varying percentage of contained agar *per se* did not appear to have any appreciable effect on the growth of the organism either at 19° C. or at 30° C.

The microscopic characters of the hyphae may vary somewhat according to the amount of moisture available. In vigorous cultures on freshly-made agars the marginal hyphae grow out fairly straight, with rather infrequent branches. On plates which are drying out, on the other hand, the hyphae become more flexuous and branching is more frequent. Finally in the compact tissue of cultures some weeks old irregular swollen cells may be found resembling those figured by Duggar (*loc. cit.* p. 418, fig. 3) as covering the surface of large sclerotia.

Bodies resembling sclerotia in form occasionally occur, especially as cultures dry out, but the structure of these is more compact and consists of more regular hyphae than Duggar describes for the large sclerotia occurring in nature. The surface of such bodies is, however, often covered like the sclerotia with a layer of short, irregularly branched and thickened hyphae. These sclerotia-like bodies found in cultures appear to be extreme developments of the compact, heaped up growth which occurs at low temperatures rather than special structures such as the sclerotia of *Botrytis*, etc.

Duggar notes that the violaceous pigment of the young hyphae as found in the field is soluble in acidulated water. The authors found that when the growth was reasonably well aerated, as a general rule, abundant pigment was formed and this readily diffused out into the acid gels or liquid cultures. In the latter where the submerged mycelium could be observed it usually appeared colourless, however deeply coloured the medium or the surface growth might become. In this connection it is interesting to note the statement of Faris that "very little pigment is produced after the mycelium enters the host." The absence of colour within the host tissues has also been noted by previous workers. The colour in liquid cultures was found to be unaffected by heat and to be insoluble in such solvents as ether, chloroform, etc.

With many of the liquid and some of the agar cultures rolled filter paper, and pieces of stick or glass rod, were placed in the flasks before sterilisation with a view to giving the organism an opportunity of climbing up out of the medium to produce fructifications in the free air. Mycelium grew freely upwards on these supports but in no case has any sign of fructification been observed either on such growth or in ordinary cultures.

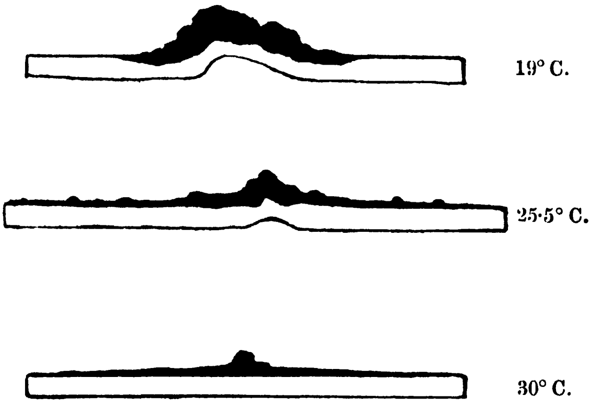
Malt agar. Growth fairly good, more brownish than purple. The mycelium piles up into compact growth several millimetres above the original level of the agar and in fresh tube cultures some loose aerial mycelium is produced. In petri dishes, where less moisture is present, the superficial growth is more even, compact and granular in appearance. In old colonies the surface growth becomes light brown, sometimes almost white. Deep brownish colour is developed in the medium, but not extending far beyond the edge of the colony. In a flask culture, after 8½ months at laboratory temperature the agar has turned a deep brown colour and the mycelial growth has become piled up into warted excrescences nearly 2 cm. in diameter. The surface of these excrescences changed in appearance from time to time, sometimes appearing deep brown, almost black, in colour and occasionally extruding drops of brownish liquid; at other times it became lighter in colour with a more pruinose appearance, and occasionally a violaceous tint. Extending irregularly over part of the surface is loose rusty brown coloured mycelium. Similar brown mycelium extends up the glass walls in places to a height of 2–3 cm.

Malt and meat extract agar. Growth good—definitely the best observed. With freshly sterilised moist agar a fair amount of loose aerial mycelium develops. The colour is variable from browns to the typical violet colour of the organism. A deep purplish or brownish colour is developed in the medium. On fairly thick layers of agar in petri dishes growth appears fairly regular and dense over the whole colony at 30° C. but the violet tinge to the brown colour is shown best at 25° C. where the growth aggregates into small rounded sclerotia-like masses 2–3 mm. in diameter. At 19° C. the growth tends to pile up at the middle of the colony with the excretion of numerous drops of dark brown liquid and is very noticeably less dense than at higher temperatures over the wide outer zone of the colony. Plate X shows the appearance of typical colonies grown at these temperatures for five weeks. "Staling" has begun—particularly at 25·5° C.—as indicated by the irregular edge of the colony. In Fig. 1 vertical sections through the middle of each of these plates are shown, in order roughly to compare the total amounts of growth. This however is only approximate, as owing to the very dark colour produced in the medium it is impossible to fix exactly the limits of the submerged mycelium.

The growth of *R. Crocorum* on malt-meat extract agar does not appear to "stale" the medium for the growth of *Penicillium* sp. as infections will grow actually on the old colony of the *Rhizoctonia.*

Malt and meat extract solution. Growth very good, commencing at once from the inoculum. It was noted that growth of a single colony per flask was no more rapid than occurred from each colony when the inoculum broke up into many pieces and many colonies were formed. The mycelium appears colourless through the bottoms of the flasks although deep colour is developed in the medium. The first aerial hyphae to appear above the surface of the solution are colourless but after an "island" of growth has been built up the surface mycelium varies in colour from brown to purple. On filter paper supports growth is poor, but on wood it spreads until the whole block is covered with a purple-brown felt—compact above but showing considerable loose mycelial development near the surface of the liquid. Eventually small bodies resembling sclerotia (see p. 299) appear near the upper end of the wood.

Malt and meat extract gelatine acidified with citric acid. Growth good with development of an intense purple colour in the medium. At the top delicately violaceous close aerial mycelium is produced. The gelatine is slowly liquefied. In some tube cultures started during a spell of hot weather the inoculum became soon submerged owing to the melting of the gelatine and in these cases no colour developed until the mycelium grew up to the aerated zone. In a flask culture started on 23rd May, 1923, and kept in a rather cold laboratory the growth was as above at first—good, with the development of much colour and some purple aerial hyphae and slow liquefaction of the gelatine. After a week or two the whole of the gelatine melted during some unusually warm weather and then remained continuously liquid with the growth submerged. All the liquid steadily developed a very intense brownish purple colour. After some eight months from inoculation an "island" of growth 3–4 cm. in diameter appeared which was opaque white, but which gradually turned to a violaceous colour. Seen through the bottom of the flask the submerged mycelium appeared colourless.



Black = visible compact fungus tissue. White = agar, more or less coloured.

Fig. 1. Drawings to show the comparative amounts of growth over the surface of the colonies of *R. Crocorum* grown for five weeks at the temperatures indicated. The lengths of sections indicate the comparative diameters of the colonies. The black indicates the amount of visible very compact fungus tissue. At 19° C. the growth over the outer zone of the colony was too thin to be indicated exactly proportionally.

Meat extract agar. Growth similar to, but not so abundant as, that on malt or on malt and meat extract agars. On thin layers of agar in plates which begin to dry out before growth has advanced very far a tendency to form the compact sclerotia-like bodies mentioned above has been observed. The colour developed in the agar is more distinctly purple usually than when malt is present.

Jardox extract gelatine. Flask culture. Growth very slow, almost entirely submerged with only slight development of aerial mycelium. Gelatine is very slowly liquefied. After eight months at low laboratory temperatures the liquefied gelatine has a colour somewhat similar to that of a prune decoction. The mycelium appears colourless and the pigment developed in the gelatine has not extended into the nonliquefied portion.

Jardox extract broth. Very slow growth, the liquid turning deep brown.

Bouillon gelatine (not neutralised)[1]. Incubated at 19° C. there is no liquefaction of the medium in 3–4 weeks. At 22–23° C. there is slight liquefaction in the centre of the colony. Growth is fairly good but there is comparatively little pigment production. The main part of the colony is reddish brown in the medium with a practically colourless outer zone. Some practically white aerial mycelium is developed in the middle.

Prune agar. At laboratory temperatures growth is slow and of a dingy purple colour. Old cultures show a compact somewhat granular looking surface layer with practically no fluffy aerial hyphae. On freshly made agar incubated at 22–23° C. growth is much better and equal to that on malt agar. The mycelium is dark purple in colour, and the colony is surrounded by a deep claret-coloured zone of diffused pigment.

Soft acid cherry decoction agar. Fair growth. After seven months in flask culture a deep brown colour extended about 3 cm. deep into the agar. Some close "woolly" aerial mycelium, very light brown in colour, was developed, but over two-thirds of the surface it was so scanty that the dark colour of the agar showed through.

Quaker Oat agar. Growth good. Aerial hyphae short and tending to form minute light brownish clusters which were at first taken for an impurity.

Thin oat agar. Growth very slow. Eventually there is a fair amount of pale cinnamon-coloured aerial mycelium and a deep red-brown colour is developed in the medium.

Corn-meal agar. Growth exceedingly slow and scanty, scarcely visible after four weeks at 22–23° C. A deep violet colour diffuses from the inoculum into the fresh medium.

Potato agar. Very little superficial mycelium; moderate growth in the agar developing to a very deep brown colour. In plates incubated at 22–23° C. the colony shows at an early stage an irregular fan-like development.

Sterilised potato plugs. Growth very slow. The surface of the block becomes gradually covered with a deep brownish felt, with very little erect aerial mycelium. Eventually the mycelium extends into the cotton wool on which the block rests. Very old cultures show the potato completely penetrated by the mycelium and small aggregations of deep brownish mycelium here and there over the surface.

Steamed rice. Growth slow, with dull reddish mauve colour developed in the matrix.

Bread paste. Similar to growth on steamed rice, eventually the whole matrix being penetrated by mycelium.

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[1] This was adapted from the medium used by Biourge (1923) for cultivating species of *Penicillium* and was prepared according to the formula: water 1000 c.c., Lemco 10 gm., peptone 10 gm., sodium chloride 5 gm., gelatine 120 gm., pure glycerine 24 gm., cane sugar 50 gm., cleared with white of egg.

Starch agar. Growth very slow with little superficial mycelium. The typical violet colour of the organism diffuses into the agar.

Dox's agar. Very slow and almost entirely submerged with only slight aerial growth: deep colour production in the agar. After ten months a considerable number of golden yellow crystals were found on the surface of the agar.

Fermi's solution and Uschinsky's solution. Practically no growth.

Milk. The inoculum sank and although there was a fair amount of growth after nine months comparatively little colour developed. The culture appeared almost white at the base with the remainder a dirty brown colour. The top ¼ inch remained liquid but below was an almost solid plug of growth. In a litmus milk tube growth occurred, by chance, at the surface and under the aerated conditions intense colour developed similar to that with the acidified malt and meat extract gelatine. The small amount of compact aerial mycelium was light violaceous in colour with some extruded drops.

Manure extract agar. Poor growth almost entirely submerged.

Peptone agar. (An acid medium developed by Waksman(10) for counting soil fungi.) Moderate growth in the agar with production of dark brown colour. In a freshly sterilised tube there was a fair amount of light brown aerial mycelium.

Clover leaf and stem extract. Very moderate growth.

Clover leaf and stem extract agar. Growth slow, not piling up above the surface at all. There is a slight development of aerial mycelium over the whole colony, giving a delicate violaceous tint viewed from above.

Sterilised clover stems. Growth very poor and soon ceased, probably owing to rather dry conditions.

Steamed oat straw and steamed wheat straw. Very slight growth.

Crocus corms. (Not *Crocus sativus*, but the ordinary English garden Crocus.) On corms which had been sterilised in mercuric chloride solution and washed with sterile water no growth at all occurred when inoculated and placed in moist chambers. This result may have been influenced by the degree of maturity of the corms, which were specially lifted early for use, or it may have been due to the species of Crocus used. On sterilised Crocus corms growth was slow and very light coloured at first but fairly abundant after ten weeks at 30° C., forming dark brown hummocks on the surface of the corms and appearing rusty brown in colour where it extended to the sides of the tubes. The brown colour extended throughout the moist cotton wool plugs at the base. Chopped up Crocus corms used for the preparation of a Crocus decoction were also sterilised in a flask and inoculated. On this rather poor nutrient material growth was very slow and chiefly of a rusty brown colour.

Crocus agar. Moderate growth, very deep brown with little aerial mycelium.

Thick Crocus decoction. Fair growth, liquid almost black, aerial mycelium rusty brown.

THE OPTIMUM TEMPERATURE FOR GROWTH IN CULTURE.

When first isolated growth on all media and at the temperatures tried appeared to be extremely slow. At summer laboratory temperatures sufficient growth for making sub-cultures was obtained only after several weeks. It was desirable to obtain some rough idea as to the optimum temperature for growth of the fungus, but before our experiments yielded reliable figures the slowly growing organism also served to emphasize the importance of the "internal condition" of fungi used in such physiological determinations. Owing to the slowness of growth of the *R. Crocorum* in our original transfers and the impatience of the authors the first attempts at obtaining some idea of the temperature relations of the fungus were made by inoculating poured plates of media with pieces of growth taken from a tube culture. The toughness of the growth and the paucity of the margin of advancing mycelium make it difficult to transfer any number of approximately equal sized pieces of mycelium or without varying quantities of the medium attached. The results obtained were most irregular, especially when a portion of agar not already thoroughly permeated by the mycelium was transferred. In that event frequently some considerable time elapsed before the fungus grew out from the inoculum on to the fresh medium—in one extreme case not doing so until after the lapse of 45 days.

It may be mentioned here that in the course of the work it has been noted also that the time that elapses before new growth starts in a sub-culture depends on the age of the culture from which the inoculum is taken. If a culture about six months old is used as parent it may be two or three weeks before any new growth can be detected. On the other hand, transfers from the growing margin of a comparatively recent culture (6–8 weeks) grow out almost at once, especially if a very small piece of inoculum is used and so placed that the mycelium is immediately in contact with the fresh food material.

Edgerton (12) in his work on the temperature relations of various strains of *Glomerella*—a comparatively rapidly growing fungus—recognised the error arising from differences in the "internal condition" of the portions of mycelium used as inocula. Using "a small bit of mycelium from the edge of a rapidly growing colony" he states that in taking his measurements "the growth of the first 24 hours was never considered." In our experiments with *R. Crocorum* the error due to this cause was so considerably magnified that, as mentioned above, using transfers of pieces of mycelium from a tube culture duplicate plates varied so much as to make the results quite worthless. Examples of such irregular duplicates—each pair being the diameters of colonies in millimetres after the lapse of the same interval of time and with other conditions equal, but the inocula being different portions from the same stock culture tube—are as follows: 26 and 53; 34 and 48; 9·5 and 28; 16·5 and 29. When, however, more elaborate technique was adopted perfectly consistent results were obtained. The figures given in Table I illustrate the good agreement that was obtained on measuring the diameters of colonies on series of similar plates. Plate X further shows the regular nature of the growth in the colonies although by the time these photographs were taken (five weeks after inoculation) "staling" had set in and the margins of the colonies were becoming irregular.

Table I.

Diameters of colonies in millimetres 14 *days after inoculation, on meat-malt extract agar.*

(Each figure is the average of two measurements to the nearest ½ mm. in directions at right angles.)

Incubated at 19° C.	20	19·45	17	17	18·25	18·25	18·25	17·5	Av. 18·2
25·5° C.	24·75	22	22·5	23·75	22·75	24·25	25·25		Av. 23·6
30° C.	18·75	21	22·25	18·75	20·75	20·75	21	20·5	Av. 20·5

Meat-malt extract agar was used as this was found to give the best growth of any of the media tried. In order to obtain a moderately deep layer of agar 30 c.c. were used to a petri dish 4 in. in diameter—the dishes being a good flat-bottomed sample. The method of inoculation was that used by Fawcett(11), equal sized slabs 2·5 × 3 mm. in diameter being cut with a Keitt platinum cutter round the edge of a regularly growing colony in a petri dish and one of these slabs being inverted on the middle of each fresh plate of medium. The fungus grew out on to the fresh agar in all cases practically at once and it was not necessary to discard any of the plates as possibly giving erroneous measurements. Table II shows the average increases in the diameters of colonies for successive weekly periods with one set of plates incubated at the temperatures given. Each colony was measured in two directions at right angles to the nearest ½ mm. and each figure is the average for seven or eight plates at each temperature. The laboratory temperature to which the one group of plates was submitted was rather low and variable, ranging from 7° to 15·5° C. and averaging about 11° C. over the 24 hour day and night period. A set of colonies which had been grown at a temperature of 30° C. for 3–4 weeks was transferred to an incubator at 37–40° C. No growth at all took place at this temperature and the young hyphae appeared to be killed by the exposure as on transferring to 25° C. no growth took place at the edges of the colonies. After 10 days at 25° C., however, a striking growth of almost colourless hyphae was taking place from the middle zones of practically all the colonies. This greater resistance of the median zones of the colonies seems to agree with the results of attempts at isolation—the more resistant and viable portions of the fungus being found to be the sclerotium-like bodies.

Fig. 2 shows graphically the relation between the diameters of the colonies and the temperatures at which they were grown at times three and four weeks after inoculation of the plates.

A second set of plates was started on meat-malt extract agar on a batch of medium which had been prepared at a different time from that used for the above and the inocula were from a different secondary stock culture plate. There were nine or ten plates in the group for each temperature and the results obtained were exactly similar to those given in Table II for the first set. The average figures for this second set are shown graphically in Fig. 3. It is regretted that with the limited amount of apparatus at the disposal of the authors it has not been possible to obtain the growth figures at definite 10° C. intervals so that no temperature coefficients can be calculated.

It will be seen from the results that at all temperatures, except the low variable laboratory temperature, the rate of growth increased with each seven-day period until the end of the third week from inoculation. The rate of growth then steadily decreased, the decrease being most rapid and the "staling" most marked at 25·5° C.

Table II.

Average increases in diameter in millimetres for successive seven-day periods of colonies of Rhizoctonia Crocorum *grown at the temperatures indicated on meat-malt extract agar in moderately deep equal layers in petri dishes.*

(Each figure is an average from seven or eight dishes and the diameter of each colony was measured in two directions at right angles to the nearest ½ mm. 2½ × 3 mm. was deducted for the size of the slab of the original inoculum.)

							Average total increases in diameter of colonies from time of inoculation (see Fig. 2)	
Temperature	1st period	2nd period	3rd period	4th period	5th period	6th period	In 3 weeks	In 4 weeks
7–15·5° C. Av. 11° C.			5·9*	5·8	8·1	8·5	5·9	11·7
19° C.	3·6	11·8	13·6	12·8	11·65	10·45	29·0	41·8
25·5° C.	5·1	15·7	16·9	15·1	9·4	7·35	37·7	52·8
30° C.	4·9	12·8	14·4	12·3	10·75	lost	32·1	44·4
37–40° C.	Nil, after developing for 3–4 weeks at 30° C.						—	—

The results of a second similar set are shown graphically in Fig. 3.

* Total of first three periods.

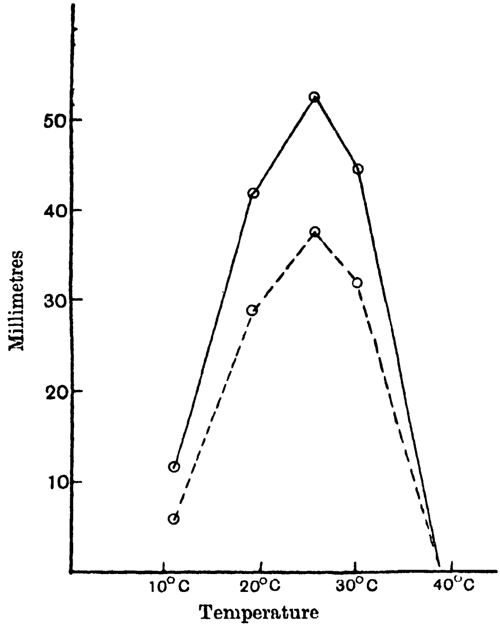


Fig. 2. Graph showing the comparative increase in diameter of colonies of *Rhizoctonia Crocorum* at different temperatures on meat-malt extract agar in three weeks – – – – – – and in four weeks ———————— from inoculation. (The temperatures for the values indicated by points at 11° C. and 39° C. are approximate only.)

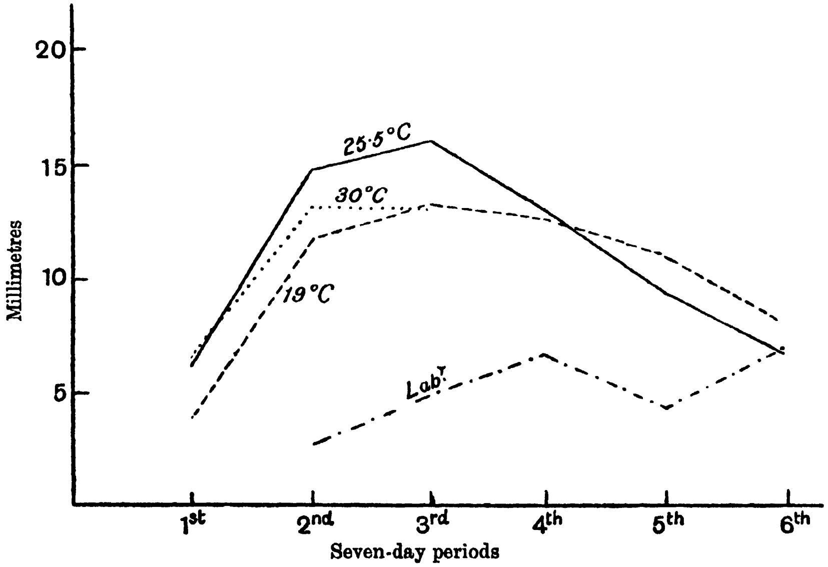


Fig. 3. Graph showing the increase in diameter of colonies of *Rhizoctonia Crocorum* for consecutive seven-day periods from the time of inoculation at the temperatures given.

(The readings for 30° C. after three weeks were not obtained in this set.)

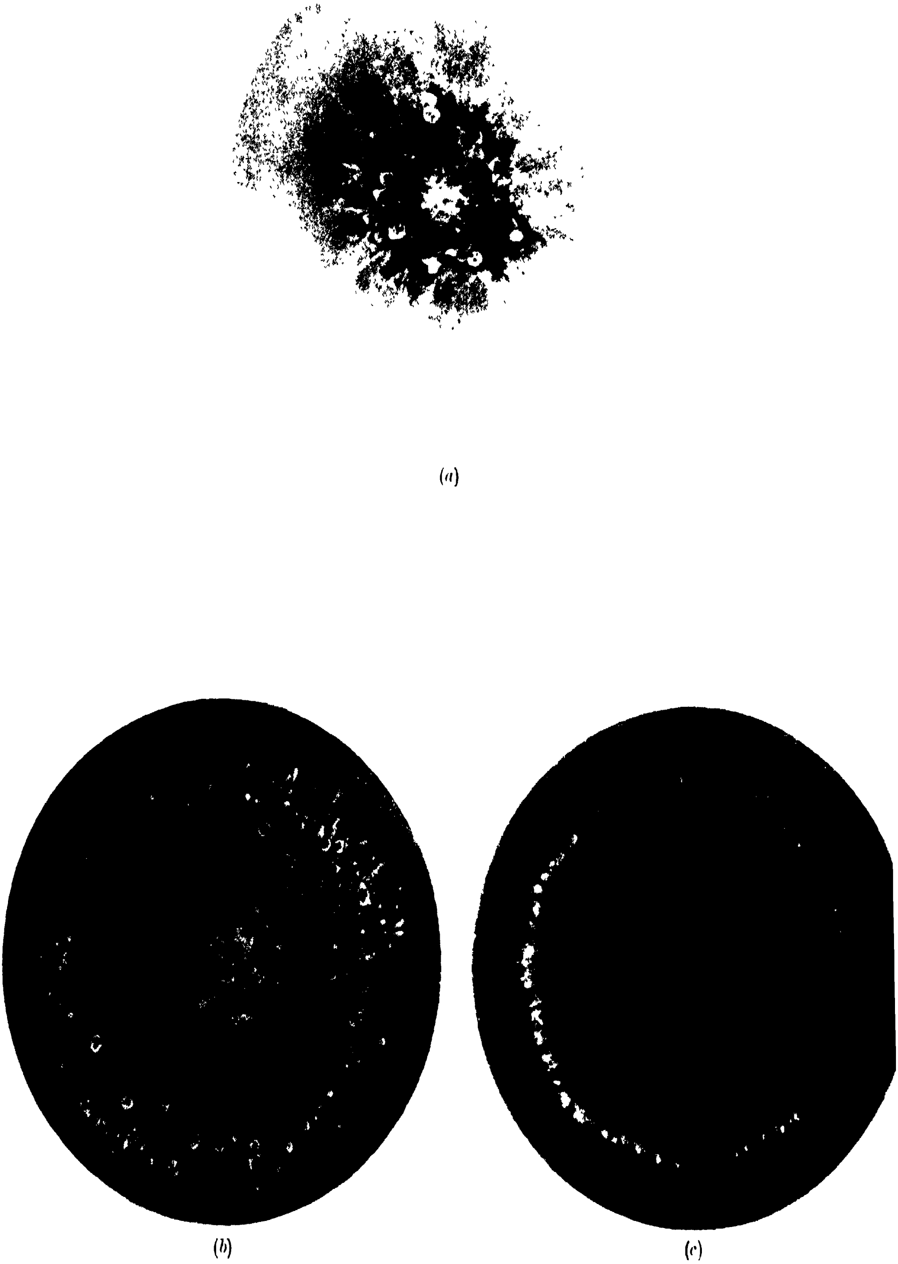
Low lab. temp. (av. 11° C.) – • – • – • – • – • – •   19° C. – – – – – – – – – – – – – –

25·5° C. ————————   30° C. . . . .

Working with his culture contaminated by a *Fusarium* Diehl states: "There was no apparent relation of various temperatures to this culture as exhibited in forty days growth on 'radicicola' agar, Thaxter's potato hard agar, and alfalfa root plugs at 10°, 20°, 22° to 25°, 30° and 37° C." On the other hand, Faris, working with potato plants grown at 90–100° F., obtained good growth of the *R. Crocorum* from infected "sets" whereas plants grown at 60–70° F. did not develop the disease. He states: "there is some indication that the fungus develops best at high temperatures." Our results given above show definitely that the optimum temperature for growth of *R. Crocorum* in culture, as measured by the increase in the diameter of colonies on meat-malt extract agar plates, lies in the neighbourhood of 25·5° C.—possibly a degree or so higher if, as is likely, the fungus shows a smooth temperature-growth curve similar to that given by other organisms of which more complete records can be found in the literature (cf. Fawcett).

Previous writers have attempted to correlate the occurrence of the disease in the field with the probable moisture relations of the fungus. Thus, according to Duggar, du Hamel represented the violet root fungus as selecting dry localities; the Tulasne brothers reported that while wet weather may give the fungus an advantage, it is found in the driest situations permitting crop growth; the fungus occurs very generally in southern Europe, especially in southern France and Italy, and in the United States it has been reported largely from regions of lower humidity and lesser rainfall. The temperature of 25·5° C., which has been shown to be the optimum for active growth of the organism, is high for a field parasite in this country, and, indeed, it is rare to find more than a few plants attacked in very limited areas in the field here. While, no doubt, moisture plays an important part in affecting the activities of the parasite, the authors are of the opinion that temperature is probably the most important factor in governing the relative abundance of the fungus in the various countries from which it has been reported.

The more definite knowledge concerning the temperature relations of *R. Crocorum* which has now been obtained is of importance in that it will allow of a more satisfactory interpretation of the results of cross-inoculation experiments and a sounder determination of the host range of the organism.



(a)

(b)

(c)

BUDDIN & WAKEFIELD.—Some Observations on the Growth of *Rhizoctonia Crocorum* (Pers.) in Pure Culture. (pp. 292—309.)

SUMMARY.

The organism dealt with in this paper is that which has been commonly known in this country as *Rhizoctonia violacea* Tul. but until satisfactory proof is forthcoming of a spore stage of the organism it should be known by its valid prior name, *Rhizoctonia Crocorum* (Pers.) DC.

The methods used for isolation of the fungus in pure culture are described and some account given of preliminary inoculation experiments with the re-isolation of the parasite.

It is shown that growth of *R. Crocorum* will occur on a very wide range of sterilised artificial material when in pure culture.

No spore form has occurred in any of the large number of cultures which have been grown.

Some notes are given on difficulties arising in temperature relation work from differences in the "internal condition" of the organism under varying conditions, and it is shown that the optimum temperature for the growth of the organism in culture on meat-malt extract agar as measured by the increase in diameter of colonies is in the neighbourhood of 25·5° C. This optimum temperature probably explains the geographical distribution of the severe attacks of the organism in the field.

REFERENCES.

(1) TULASNE, L. R. and C. (1851). *Fungi Hypogaei.* Paris. p. 188.
(2) DUGGAR, B. M. (1915). *Ann. Mo. Bot. Gard.* II, pp. 403–58.
(3) DIEHL, W. W. (1916). *Phytopath.* VI, pp. 336–40.
(4) VAN DER LEK, H. A. A. (1917). *Meded. v. de Rijks Hoogere Land-, Tuin-, en Bosch-bouwschool.* Wageningen. Tome XII.
(5) FARIS, J. A. (1921). *Phytopath.* XI, p. 413.
(6) WARE, W. M. (1923). *Journ. Min. of Ag.* XXX, p. 48.
(7) MANNS, T. F. (1914). *Delaware Ag. E.S. Bul.* 108.
(8) KLEBAHN, H. (1918). *Haupt- und Nebenfruchtformen der Askomyzeten.* Erster Teil. Leipzig.
(9) ERIKSSON, J. (1915). *Arkiv för Bot.* 14, No. 12, pp. 1–31.
(10) WAKSMAN, S. A. (1922). *Soil Science,* XIV, pp. 28, 156.
(11) FAWCETT, H. S. (1921). *Univ. of California Publ. in Agric. Sci.* IV, pp. 183–232.
(12) EDGERTON, C. W. (1915). *Phytopath.* V, p. 249.

EXPLANATION OF PLATE X.

Photographs showing the appearance of colonies of *Rhizoctonia Crocorum* after growing for five weeks at (*a*) 19° C., (*b*) 25·5° C., and (*c*) 30° C. (Nat. size.)

Photos by G. ATKINSON.

(*Received May 10th,* 1924.)

THE FUNCTION OF LINT HAIRS IN THE LIFE HISTORY OF THE COTTON PLANT

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CONSIDERING the importance of raw cotton and the amount of attention it has received from all branches of science it is rather remarkable that a really satisfactory explanation of the natural function of lint hairs has not yet been put forward.

Haberlandt and others consider it as a feeble means of seed dispersal. Denham[1] remarks that no explanation is necessary and makes the unique suggestion that the lint is an outlet for excess polysaccharides. This would be the first instance on record of excess food material being stored in such a manner as to be unavailable for future use.

The lint cannot be of any value to the cultivated plant since it is removed immediately after maturation when it should reasonably be expected to assume its functions. Balls[2] observed in Egypt that self-sown cotton plants showed a better chance of survival than ginned seeds under the same conditions of water shortage. Actual germination tests however show that the seeds germinate quicker after de-linting and give a higher percentage germination. To argue from this that the lint has no useful function to the plant in the natural state shows a lack of appreciation of the peculiar character and adaptation of the cotton plant. Besides it is hardly a comforting thought that such an important industry rests on a biological freak.

The specialised types of cotton possessing high yield of lint of long staple (e.g. *Gossypium barbardense*) are annuals of rapid growth and early maturity and appear to grow best in a climate of well-marked wet and dry seasons. In other words, their natural habitat is one requiring xerophytic characters, yet the cotton plant is remarkably free from such characters and is exceedingly sensitive to soil moisture conditions and solar radiations. Under cultivation the crop is sown towards the end of the rainy season and as the dry season advances a very extensive root

[1] *Shirley Institute Memoirs*, I, 1922.
Development and Properties of Raw Cotton, 1915.

system is rapidly developed to keep in touch with the receding water table. Any subsequent abnormal rise in the water table is attended with damage to the crop.

In a wild state the seeds would require protection from temporary changes in humidity such as might occur with heavy dew or sporadic rainfall that might initiate germination without provision for subsequent growth. For this purpose a thick covering of cotton lint is eminently suitable. Also the lint being rolled about by the wind during the dry season, would collect round it the débris of dead leaves, etc., and ultimately come to rest in some hollow or sheltered position. With the advent of the definite rainy season humification of the mass of lint and débris would have set in before germination occurred.

Thus the evolution of lint hairs may be considered a special adaptation to climatic conditions. Even to-day, notwithstanding the artificial extension of cotton areas, the varieties with most highly developed lint (*e.g.* Sea Island and Egyptian) are to be found only in climates with well-marked regular seasons whilst the types with more fuzzy types of lint are found in more erratic climates.

(*Received April 2nd*, 1924.)

STUDIES IN BACTERIOSIS

XI. A BACTERIAL DISEASE OF LETTUCE

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(With 2 Text-figures.)

This disease of lettuce occurred during the summer of 1921 and the extent of the damage caused is not known; the material was submitted for examination by the Ministry of Agriculture and Fisheries. It came from a private garden where 75 per cent. of the plants were attacked. The disease shows itself by a browning of the leaves beginning usually at the margins and working inwards; the veins are first attacked and appear as a fine brown network; the attacked tissue becomes the prey of saprophytic bacteria and moulds and is quickly reduced to a wet rotting mass. From portions of the leaf in which only the veins were attacked an organism was isolated and obtained in pure culture without much difficulty. This organism produced the disease when deposited on the surface of healthy lettuce either by spray or brush inoculation. A photograph of the original diseased plant appears in Fig. 1 and the result of one of the infection experiments is shown in Fig. 2.

Description of the Causal Organism.

A. *Morphological Characters.*

The organism is a bacterium with polar flagella, one to three at one pole (a few cases were noted with five at one pole). Caesares-Gil's Flagella stain was used to demonstrate the flagella. The organism is a short rod with rounded ends occurring singly or in chains.

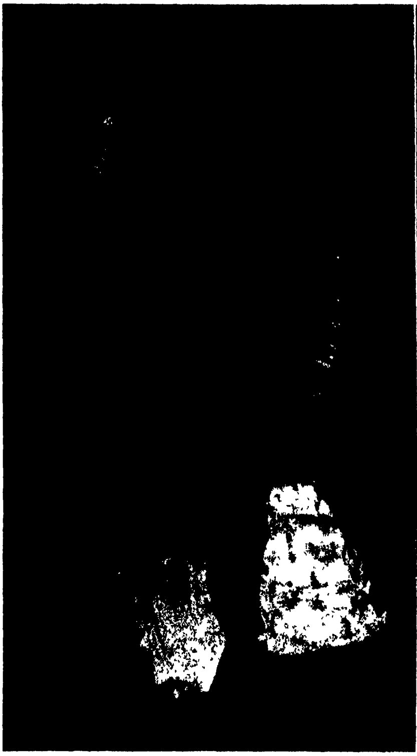
Stained with carbol fuchsin the organism measures $1{\cdot}6\mu$ to $2{\cdot}2\mu$ long and $0{\cdot}8\mu$ to $1{\cdot}1\mu$ wide, the average size being 2μ long by $0{\cdot}9\mu$ wide.

Stained with gentian violet the organism measures 2μ to $1{\cdot}3\mu$ long by 1μ to $0{\cdot}5\mu$ wide, the average size being $1{\cdot}6\mu$ long by $0{\cdot}8\mu$ wide.

The staining and measuring were carried out on 18–24 hour old cultures on bouillon agar.

Capsules were suspected, and eventually a successful preparation showing them was made with Ribbert's capsule stain.

Endospores were not observed. Tests for spores were made by heating old bouillon cultures of various ages to 80° C. for 20 minutes. Transfers were made in each case before and after heating, but no growth was obtained in cultures made by transfers after such treatment, although before heating the cultures were alive.



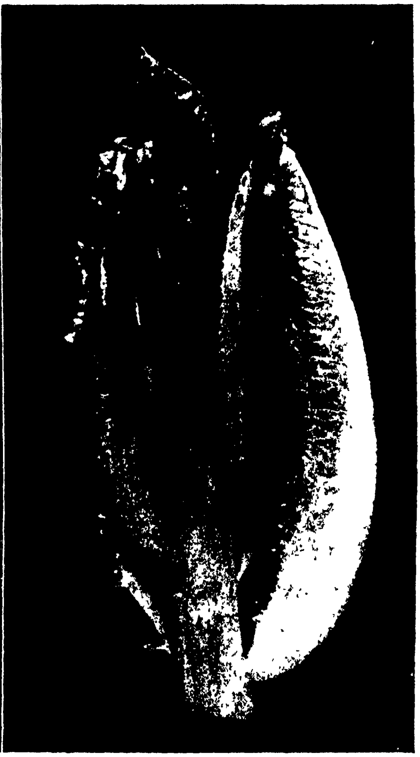


Fig. 1. Showing a diseased lettuce plant. Fig. 2. Showing the result of inoculation.

Behaviour towards Stains. The organism stains readily with carbol fuchsin, gentian violet, methylene blue, victoria blue and bismarck brown. It is Gram negative and not acid-fast.

B. *Cultural Characters.*

The organism grows readily in most media, but growth is most rapid in bouillon (*p*H 6·8–7) at 30° C.

In the following series of experiments the temperature of incubation was 25° C. unless otherwise stated.

B. Agar Plates (*p*H 6·7). The colonies on poured plates appear after 24–28 hours. Surface colonies are 1½–2 mm. in diameter after two days, later they vary in size from 2–5 mm. Depth colonies are lenticular, and vary from 0·5–1 mm. in diameter. The surface colonies are round, convex and wet-shining. The colour is ivory-yellow[1] by reflected light, and cartridge-buff by transmitted light. The medium is at no time stained by the organism. Microscopically the colonies appear granular and somewhat denser at the centre.

Streak Culture on B. Agar (*p*H 6·8). Growth is visible after 24 hours. In three days the growth is thin, spreading, wet-shining, and ivory-yellow in colour. No staining of the medium is visible. The culture smells decidedly, the smell resembling that of rotting cabbage.

Stab Culture in B. Agar (*p*H 6·8). Growth is visible on the surface after two days, the colony being 4 mm. in diameter. After eleven days, the surface colony is 1 cm. in diameter, and is flat, thin, entire and of a cream-buff colour. The medium is not stained. Growth is limited to the surface.

Plate Culture on B. Gelatine (*p*H 6·8). Colonies become visible in 24 hours at air temperature, and the gelatine is partially liquefied. In three days the plate is entirely liquefied. The colonies are 1 mm. in diameter.

Stab Culture in B. Gelatine (*p*H 6·8). Liquefaction begins as a crateriform depression in 1–2 days at air temperature, and there is slight growth visible in the upper part of the stab. In eleven days, the liquefied portion of the medium is 7 mm. deep, stratiform, with a heavy precipitate. After six weeks, the gelatine is not entirely liquefied, the solid portion occupying about 1·5–2 cm. at the bottom of the tube.

Streak Culture on Cooked Potato. Growth is visible after 24 hours. The streak is thin, spreading, and honey-yellow in colour; in eleven days, the potato medium has darkened slightly.

Nutrient Bouillon Broth (*p*H 6·8). Strong clouding of the medium takes place in 16–24 hours at 25–30° C. After eleven days, the medium is persistently turbid, with a compact sediment which is viscid on agitation; the medium is naphthalene-yellow in colour with very slight fluorescence; the tubes have a decided odour, which resembles rotting cabbage.

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[1] The colours mentioned in this paper are given according to Ridgway (Ridgway, Robert, *Color Standards and Color Nomenclature*, 43 pp., Col. Pl. Washington, D.C., 1912).

C. *Physiological Characters.*

For the following tests a 48-hour old culture in bouillon was used for inoculating the tubes, which were afterwards incubated at air temperature (15-20° C.).

1 *per cent. Glucose and Peptone Broth.* Growth is visible after 24 hours, and the litmus shows an acid colour in two days, and there is a slight pellicle. After four weeks the medium is onion-skin-pink in colour with a congo-pink precipitate; no gas is formed, and no clouding takes place in the closed limb of the Einhorn tube.

1 *per cent. Lactose and Peptone Broth.* Growth is visible in two days, but no change in the colour of the medium takes place; a slight pellicle is formed after eleven days; there is no change in appearance of the tube at the end of two months; no gas is formed, and no growth takes place in the closed limb of the tube.

1 *per cent. Sucrose and Peptone Broth.* Growth is visible after two days and in four days the litmus shows an acid reaction in the upper part of the tube; after four weeks there is a corinthian-red pellicle and the upper part of the medium is salmon coloured, while the lower part is bleached; there is no gas formation, and no growth takes place in the closed limb of the tube.

1 *per cent. Maltose and Peptone Broth.* There is clouding and a slight pellicle after eleven days; after six weeks the tube shows no colour change, and no gas is formed.

1 *per cent. Mannite and Peptone Broth.* There is clouding of the medium after eleven days, but no change in colour of the medium is visible; after six weeks the tube shows no change, and no gas is formed.

1 *per cent. Peptone Broth.* The same result as with mannite above.

1 *per cent. Nitrate Broth.* Growth is visible in the tube after four days. There is no reduction; tests for nitrite using α-naphthylamine-sulphanilic acid were made weekly for six weeks and consistently gave negative results.

Potato Plug. About 1 c.c. volume of plug was taken and ground up in 200 c.c. of water. The addition of 1 c.c. of iodine solution produced a blue violet, which was not so intense in colour as in the case of the control plug but the difference was not sufficiently marked to indicate diastatic action.

Uschinsky's Solution. The medium is turbid on the second day; in eleven days there is a heavy pellicle with long filaments hanging down into the medium, which shows fluorescence and is light-lumière-green in colour, and very turbid.

Cohn's Solution. There is no growth in this medium; at the end of six months the medium in the tube is quite clear.

Litmus Milk. A slight reddening of the litmus in the top layer of the tube is visible in two days; this reddening gradually spreads downwards and after eleven days has reached the bottom of the tube; by this time the top centimetre of the medium is clear, but a reddish ring persists on the sides of the tube at the surface of the medium. After a month, the medium is entirely bleached, the upper two-thirds being clear, while the curd occupies the lower third.

Indol Production. Tests for indol were made by means of the Rosindol method, but negative results were obtained in all trials.

Temperature Relations. The Thermal Death Point lies between 51° and 52·5° C. The maximum temperature for growth is below 37° C. A bouillon culture kept at 37° C. for seven days shows no growth, but becomes turbid after 24 hours when transferred to the 25° C. incubator. The minimum temperature for growth is below 10° C. The lowest limit was not ascertained. The optimum temperature is 30° C.

Relation to Moisture. The organism is not very sensitive to drying. Loop transfers were made from a 19-hour old bouillon culture on to sterile strips of mica, kept at air temperature in the dark in a ventilated Petri dish. One of these strips was transferred daily to a tube of bouillon in which it was shaken up. These daily transfers were continued for 14 days in succession and in each case growth occurred in the bouillon tube after incubation for seven days at 25° C.

Vitality in Culture Media. The organism lives for six months and more in bouillon kept at room temperature (15–20° C.).

Group Number. According to the older descriptive chart of the Society of American Bacteriologists the group number of this organism is 211. 2322133.

RELATIONSHIP OF THE CAUSAL ORGANISM[1].

From the first series of cultural experiments, the organism described in the foregoing pages was suspected of identity with, or near relationship to, *Bacterium marginale*, an organism which was first isolated and described by Miss. Nellie A. Brown in America (3). To gain further information on this point, application was made to Miss Brown for a culture of *B. marginale.*

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[1] Since this paper was sent to press Dr Mehta and Dr Berridge have shown (see the following paper, p. 318) that *B. marginale* is identical with *B. pyocyaneus*, and there is no doubt that the organism here described is also a strain of this latter organism.

Miss Brown kindly supplied cultures of three organisms pathogenic to *Lactuca sativa*, all isolated and described by her. With these cultures, a parallel series of cultures of all four organisms was set up and carefully watched for six weeks. It was soon evident that *Bacterium marginale* and our organism were most nearly allied, and no further comparative study was made with the organisms *Bacterium viridilividum* and *Bacterium vitians*. Henceforth parallel experiments were confined to *Bact. marginale* and our organism. From these experiments it appears that although the organisms have many reactions in common, they are not identical.

It was noticed that the "Group Number" for our organism agreed with that given for *Bacterium aptatum*, isolated and described by Miss N. A. Brown and Miss C. O. Jamieson as pathogenic to Sugar-beet and Nasturtium leaves (1) and also with that given for *Bact. xanthochlorum* and *Ps. tenuis*. Application was made to Miss Brown for cultures of these organisms, but she was only able to supply a culture of *Bact. aptatum*. On receipt of this culture a comparative series of cultures of our organism and *Bact. aptatum* was set up. From these tests it was found that the two organisms were in essential characteristics so nearly identical that in the opinion of the writers the two are one and the same species. Any slight differences which exist may be attributed to adaptation to different hosts.

The authors desire to take this opportunity of expressing their thanks to Miss N. A. Brown for her courtesy in supplying cultures and information which have been used in this work.

LITERATURE.

(1) BROWN, NELLIE A. and JAMIESON, CLARA O. A Bacterium causing a disease of Sugar-Beet and Nasturtium leaves (*Bact. aptatum*). *Journ. Agric. Res.* IV, No. 5, 1915.

(2) BROWN, NELLIE A. A Bacterial disease of Lettuce (*Bact. viridilividum*). *Ibid.*

(3) —— Some Bacterial diseases of Lettuce (*Bact. vitians*, and *Bact. marginale*). *Ibid.* XIII, No. 7, 1918.

(*Received May* 11*th*, 1924.)

STUDIES IN BACTERIOSIS

XII. *B. PYOCYANEUS* AS A CAUSE OF DISEASE IN LETTUCE AND THE IDENTITY OF *B. MARGINALE* WITH THIS ORGANISM

By MANECK M. MEHTA and EMILY M. BERRIDGE.

(From the Department of Plant Physiology and Pathology, Imperial College of Science and Technology, London.)

(With Plate XI.)

In 1918 a description of two species of bacteria which attack lettuces, and had been the cause of considerable loss to market gardeners in America, was published by Miss N. Brown (1). Sub-cultures of these were received in 1922 by Dr S. G. Paine from Miss Brown for purposes of comparison with an organism isolated from diseased lettuces grown in England.

One of these organisms, *B. marginale*, has, since then, been kept in this laboratory, mainly on bouillon agar, and has been regarded as a typical plant parasite of the Pseudomonas type. Its power to produce green coloration of the medium has become more and more marked, so much so as to suggest the possible identity of this organism with *B. pyocyaneus*.

One of us (M. M.) had previously been engaged in a careful redetermination of the morphological and cultural characters of a series of bacterial species; these included *B. marginale* and a strain of *B. pyocyaneus* obtained from the Lister Institute. When the records for these were compared they were found to be identical in every respect and are given in Table I.

These redeterminations of the characters also correspond very closely with those of Miss Brown's original description of the species, proving that it had not altered materially in the course of the two years it had been kept in this laboratory. One or two discrepancies certainly are to be found; for instance, Miss Brown states that *B. marginale* produces acid in saccharose after six weeks, whereas we concluded that no acid was formed since our cultures in saccharose rapidly became green and remained so for three months, and this green colour is in itself an indication of the alkalinity of the medium.

The characters given in Table I also agree with those given by Lehmann and Neumann (2) for *B. pyocyaneus*, except that our strain of the organism was Gram-negative, as was also *B. marginale*. Tromsdorf (3), however, who studied 27 strains of *B. pyocyaneus*, found that some strains of the organism were Gram-negative.

Table I.

Showing the close agreement of B. marginale *and* B. pyocyaneus *in their morphological and cultural characters.*

<table>
<tr><th></th><th><i>B. marginale</i></th><th><i>B. pyocyaneus</i></th></tr>
<tr><td>Spores</td><td colspan="2">None</td></tr>
<tr><td>Aerobism</td><td colspan="2">Markedly aerobic
? Facultative anaerobe—very slight growth observed</td></tr>
<tr><td>Liquefaction of gelatine</td><td colspan="2">Rather slow liquefaction</td></tr>
<tr><td>Glucose broth</td><td colspan="2">Acid, no gas, no green colour</td></tr>
<tr><td>Lactose broth</td><td colspan="2">No acid, no gas, peacock-green colour
Ring and pellicle on the surface on the 3rd day</td></tr>
<tr><td>Saccharose broth</td><td colspan="2">No acid, no gas, peacock-green colour
Ring and pellicle on the surface on the 3rd day</td></tr>
<tr><td>Nitrate broth</td><td colspan="2">Good growth, much gas after 24 hours
Strong reduction, nitrate and nitrite has all disappeared by the 15th day</td></tr>
<tr><td>Chromogenesis</td><td colspan="2">Yellow-green turning to bluish-green as the alkalinity of the medium increases</td></tr>
<tr><td>Growth on potato</td><td>Greenish-buff in 48 hours</td><td>Sometimes yellow, sometimes greenish-buff in 48 hours</td></tr>
<tr><td>Diastase reaction</td><td colspan="2">Very slight, if any</td></tr>
<tr><td>Morphology</td><td colspan="2">Very slender rods, rounded at the ends, actively motile by one polar flagellum, capsulated</td></tr>
<tr><td>Size</td><td>2·4–1·5μ × 0·6μ</td><td>2·7–1·5μ × 0·5μ</td></tr>
<tr><td>Gram-stain</td><td colspan="2">Negative</td></tr>
<tr><td>Thermal death point</td><td colspan="2">54° C.</td></tr>
<tr><td>Growth in broth</td><td>Good growth with yellow-green colour in 24 hours at 25° C., pellicle formed in 48 hours</td><td>Good growth with green colour in 24 hours at 25° C., pellicle on the 3rd day</td></tr>
<tr><td>Peptone water</td><td>Clouding with light blue colour in 36 hours, bluish-green colour on the 3rd day</td><td>Clouding with light green colour in 36 hours, dark green colour on 5th day turning salmon-pink after a fortnight</td></tr>
<tr><td>Indol</td><td colspan="2">Could not be determined, as any acid, <i>e.g.</i> a few drops of dilute hydrochloric acid, gives a pink colour to the culture</td></tr>
<tr><td><i>B. agar</i> streak</td><td>Dull yellow growth in 18 hours, medium turns green in 36 hours, and the growth shows a pearly lustre</td><td>Dull greenish moist growth with "erose" margin in 36 hours, medium turns green on the 4th day, and the growth shows a pearly lustre</td></tr>
<tr><td>Bouillon agar plate</td><td>Small greyish-white colonies in 48 hours. Medium turns bluish-green by the 3rd day</td><td>Small round yellowish-white colonies in 18 hours. Medium turns from yellowish to bluish-green by the 9th day</td></tr>
</table>

Table I (*contd.*).

	B. marginale	*B. pyocyaneus*
Gelatine plate	Tiny greenish-white irregular colonies in 48 hours, with cup-shaped depressions due to liquefaction. On the 3rd day general liquefaction occurs, and the medium turns dark green	Tiny greyish-white irregular colonies with granular appearance in 18 hours, with green coloration of the medium. Slight liquefaction by the colonies on the 2nd day which becomes general on the 3rd day
Gelatine stab	Filiform growth in 36 hours, stratiform liquefaction in 5 days, green colour at the surface	Cylindrical growth in 36 hours, stratiform liquefaction in 10 days, green colour at the surface
Milk	Alkaline in 54 hours; a soft clot is formed, and peptonisation begins on the 7th day. A greenish curd persists for at least 2 months	Alkaline in 54 hours; a soft clot is formed, peptonisation begins on the 3rd day. A curd persists for at least 6 weeks, and the liquid above turns green
Uschinsky's solution	Alkaline, no gas, bluish-green ring and pellicle in 18 hours, yellow-green colour on the 3rd day	

Infection experiments.

Healthy young lettuce leaves, and plants of the Cos variety from which the outer leaves had been removed, were sprayed with 24-hour cultures of *B. marginale* and *B. pyocyaneus* in "Jardox" peptone broth, and also with cultures in the same broth with 1·5 per cent. glucose added. The cultures were diluted with sterile water before spraying, and were boiled for half-an-hour before spraying the controls.

The disease first appeared as minute rust brown spots along and near the midrib, veins and margins of the leaves, particularly in the pockets where the culture suspension had lodged. The organisms evidently enter by the stomata, the cells surrounding them becoming filled with a brownish pink zoogloea, the pink colour being characteristic of *B. pyocyaneus* when growing in an acid medium. As the spots spread and coalesce, brown patches appear, particularly along the margins, and later the whole leaf becomes brown and flaccid. To obtain photographs of them in this condition (Plate XI, fig. 4) they had to be floated in water on to sheets of glass as they would not bear touching. The midrib turns a rust brown colour and later also becomes soft and pulpy.

The following table gives details of the course of the disease due to *B. pyocyaneus*, and shows that the symptoms are essentially the same as in the case of disease due to *B. marginale*; the only difference being time variations, and even these are not constant.

Lettuce Leaves.

Infection culture	Days	*B. marginale*	*B. pyocyaneus*
I. Bouillon	4	Brown patches at the apex	A few spots at the apex
	6	Brown patches all over the leaf, midrib brown, margin dead	One patch at the apex
	8	Completely withered and dead	Right half of leaf dead
	9	—	Completely withered and dead
Control with boiled broth	9	Healthy except for slight damage at the apex	Healthy
II. 1·5 % glucose broth	5	Healthy	Many tiny brown spots at apex
	10	Large brown patches on margin and lamina	Apex and margin yellow
	13	Lamina almost completely withered and dead	One half of lamina almost completely dead
	15	Dead	Dead
Control with boiled glucose broth	15	One leaf healthy. Second leaf healthy till 10th day, then fungus spot appeared at apex	One leaf healthy. Second leaf slightly spotted after the 8th day

These experiments were repeated three times with similar results.

Lettuce Hearts.

Infection culture	Days	*B. marginale*	*B. pyocyaneus*
I. Bouillon	3	Many small brown patches	No spots
	4	One leaf dead, others showing brown patches	Margin at apex brown
	6	Four leaves dead	Leaves brown and dead at the apices and along the margins
	8	Whole withered and dead	Upper halves of leaves dead
	10	—	Whole withered and dead
II. 1·5 % glucose broth	3	Healthy	Apices and margins spotted and turning brown
	4	A few brown spots at apex of leaves	Brown patches at apex of leaves
	6	Many spots	Upper part of three outer leaves dead
	8	Upper part of outer leaves dead	Upper half dead
	10	Whole dead	Whole dead
III. Control boiled glucose broth	12	Healthy, apex has grown 2–3 inches	Healthy

These experiments were repeated with similar results.

Midribs of Leaves.

Infection culture	Days	*B. marginale*	*B. pyocyaneus*
1·5 % glucose broth	4	Centre turning brown	Rot beginning on one side
	9	Rot slowly spreading	Rot spreading
	14	Rot still spreading	Quite rotten
Control with the same broth boiled	14	Quite healthy	Quite healthy

These experiments were repeated with similar results.

Stems.

Although stems were repeatedly inoculated with broad organisms, and the infection pricks in a few cases became brown and exuded a watery fluid, no general infection of the tissue followed.

Re-isolation of B. pyocyaneus *and* B. marginale.

A portion of a leaf which had turned brown four days after spraying with *B. pyocyaneus* was ground up with sterile sand and plated on agar. Pure cultures of smooth, round, yellowish-white transparent colonies were obtained on the following day, which later developed the green pigment, pearly iridescent lustre and umbonate form characteristic of the species. Inoculation of the crushed leaf into broth also gave the green colour.

B. marginale was re-isolated in the same manner from a leaf showing light brown patches nine days after spraying with a glucose broth culture of this organism. The colonies on agar plates resembled those of *B. pyocyaneus* in every respect.

Both cultures were carefully re-tested, especially for acid production in sugars. Both again gave no acid with saccharose, and the green colour appeared on the 4th day, while with glucose they gave no acid reaction, and up to the 20th day no green colour had developed.

In nitrate broth reduction to nitrite had already begun on the 2nd day, and was complete by the 15th.

The striking series of colour changes in litmus milk described by Miss Brown for *B. marginale* also occurred in the milk cultures of both organisms; they showed on the 2nd day a pink ring merging into a yellow and white pellicle while the rest of the milk below was a lilac litmus colour. Digestion began on the 3rd day, and by the 7th a green ring had appeared below the yellow pellicle and a soft pinkish curd had separated at the bottom of the tube.

It seems rather significant that this disease of lettuces occurred in America at greenhouse temperatures, on highly manured soil, and after the plants had been splashed and flooded with water. *B. pyocyaneus* is an organism frequently found in water, especially in the tropics, and is an intestinal organism able to grow vigorously at body temperatures.

SUMMARY.

B. marginale, the causal organism of a disease of lettuces described by Miss N. Brown in 1918, appears to be identical with the well-known

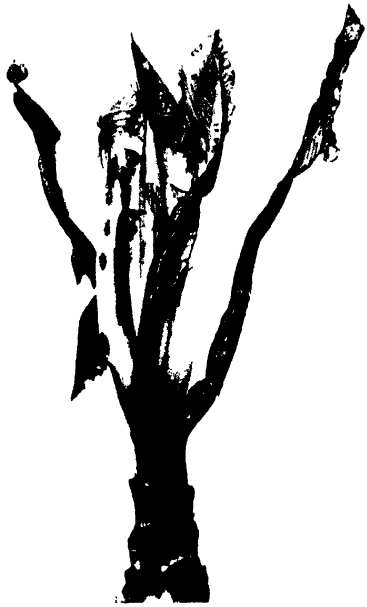

Fig. 1.

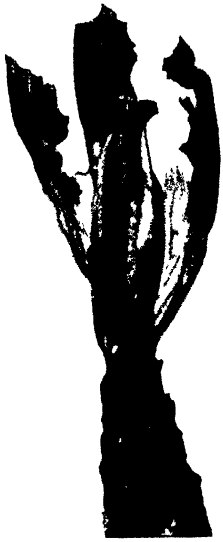

Fig. 2.

Fig. 3.

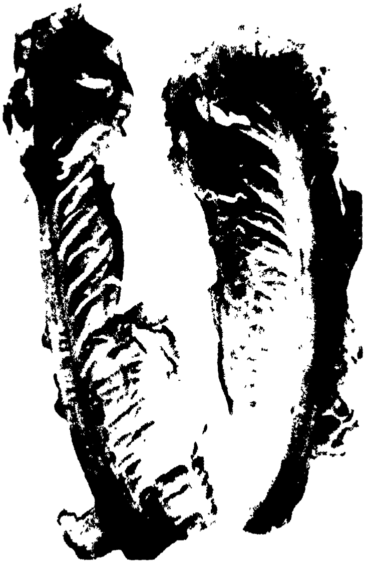

Fig. 4.

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B. pyocyaneus. They agree in their morphological and cultural characteristics, and the latter has been shown to be capable of attacking young lettuce leaves and producing disease similar to that due to *B. marginale*.

LITERATURE.

(1) BROWN, NELLIE A. 1918. Some Bacterial Diseases of Lettuce. *Journ. Agric. Res.* XIII, No. 7, p. 367.

(2) LEHMANN, K. B. and NEUMANN, R. O. *Atlas and Principles of Bacteriology.* London and Philadelphia, 1901.

(3) TROMSDORF, R. 1916. Zur Kenntniss des *B. pyocyaneum* und seiner Beziehungen zu den fluoreszierenden Bakterien. *Centralbl. für Bakt.* I. Abt. orig., Bd. 78, Heft 7, S. 493.

EXPLANATION OF PLATE XI.

Fig. 1. Heart of diseased lettuce eight days after inoculation with *B. marginale*; tissues blackened.

Fig. 2. Heart of diseased lettuce ten days after inoculation with *B. pyocyaneus*; tissues blackened.

Fig. 3. Heart of a lettuce used as control; quite healthy ten days after spraying with a boiled culture.

Fig. 4. Lettuce leaves; that on the left sprayed with *B. marginale*, that on the right with *B. pyocyaneus*. Both show spots and withered brown margin.

(*Received July 24th*, 1924.)

ON THE STIMULATING ACTION OF CERTAIN ORGANIC COMPOUNDS ON CELLULOSE DECOMPOSITION BY MEANS OF A NEW AEROBIC MICRO-ORGANISM THAT ATTACKS BOTH CELLULOSE AND AGAR

BY P. H. H. GRAY AND C. H. CHALMERS.
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(With Plates XII and XIII and 2 Text-figures.)

THE decomposition in the soil of plant residues, which are largely composed of cellulosic material, is of great importance to agriculture, and the nature of this process is at present but little understood either from its biological or its chemical aspect. Cellulose is decomposed with some difficulty and by but a limited number of organisms; the products of its decomposition appear, however, to be of great value in the economy of the soil. For example, under proper conditions of aeration, moisture, etc., crop residues give rise in the soil to the humic matter which plays a large part in crop production. Although the steps leading to the formation of this humus are at present not clearly defined, there is reason to think that further knowledge of the decomposition of cellulose under such conditions as obtain in the soil will throw light on this important question.

The earliest biological investigation, made by Omeliansky, dealt with the breaking down of cellulose by anaerobic bacteria. In normal arable soils, however, the conditions are largely aerobic, and, while the work of Omeliansky and Solunskoff (10), and of Truffaut and Bezssonoff (14), on nitrogen fixation by *Clostridium Pasteurianum* suggests the possibility that symbiosis between aerobic and anaerobic bacteria may be a factor to be considered, it seems to be of first importance that knowledge should be obtained as to the aerobic soil organisms capable of attacking cellulose, and the conditions under which they operate.

Bacteria that can decompose cellulose under aerobic conditions are known to occur fairly extensively in nature. Van Iterson (6) was the first to draw attention to this type of organism; he did not, however, obtain a pure culture of the bacteria which brought about the destruction of the cellulose. Several species have been described by Kellerman and McBeth (7) and their associates (8, 9); these workers lay especial stress on their finding that these organisms rapidly lose the power of destroying cellulose when grown under cultural conditions. Their criterion for cellulose destruction lay in the appearance of a clear zone about colonies on agar media which contained precipitated filter paper as the source of carbon. Hutchinson and Clayton published in 1919 their paper (4) describing a spirochaete-like organism to which they gave the name *Spirochaeta cytophaga*. This organism does not grow on the ordinary media containing organic substances such as peptone and meat-extract, and great difficulty was experienced in obtaining pure cultures until a dilution method was adopted. Cultures were then grown successfully on agar enclosing sheets of filter paper, and cultivated in tubes of liquid media consisting of a strip of filter paper half immersed in mineral salts solution. This appears to us to be a better criterion of cellulose destruction than that of Kellerman and McBeth, for a great many soil organisms can grow on plain mineral salts agar without any other source of energy material. Moreover, the appearance of a clear zone is sometimes produced around colonies owing to the solution of phosphates and carbonates in the medium.

In the course of purifying cultures to isolate *Sp. cytophaga*, a new type of aerobic cellulose-decomposing organism was obtained. A description of this new organism follows.

PART I.

MICROSPIRA AGAR-LIQUEFACIENS (n.sp.).

The new organism isolated has the following characters:

Source. Rothamsted soil, 9th June, 1923.

Morphology. Short curved rods, usually C-shaped, with occasional S-shaped and coccoid forms.

Size. 2 μ long by 0·5 μ to 0·7 μ broad; 3 μ to 5 μ long in division stages. Coccoid forms difficult to measure in the living state, but stained are 0·5 μ to 0·7 μ long.

Motility. Very active in young cultures. Cells remain active for some months.

Flagella. Single, polar; short, as long as or twice the length of the major axis of single cells; slightly undulating.

Oxygen Relations. Very aerobic. No growth takes place in the closed arm of the fermentation tube. In liquid cultures containing filter paper it first attacks the paper at the air-liquid level.

TEMPERATURE. Optimum 25° C. Will grow at 16° but not 34°.

AGAR SLOPE. Peptone (0·5 per cent.), Lemco (0·3 per cent.), Agar (1·5 per cent.).

In 24 hours at 25° C. a deep groove is cut along the inoculation streak, a whitish growth lining the sides. As growth reaches to its maximum the groove becomes deeper and the liquid at the bottom increases to more than twice its volume (Plate XII, fig. 2). The middle of the slope bulges outward on account of the gel being weakened. Old cultures, on being shaken, break down to amorphous lumps of agar.

1 per cent. glucose added to nutrient agar prevents the liquefaction from taking place.

AGAR COLONIES. The same medium as above.

Five days at 25° C. *Surface colonies.* Whitish growth in a depression, surrounded by a white ring. Under the half-inch objective; a brown central nucleus shading off to transparency, surrounded by a wide greyish band with an undulating inner and entire outer edge. These characters are not always visible on account of the variability in the amount of gel-weakening. Motile organisms can sometimes be seen around the outer edge, pushing their way into the zone of semi-liquefied agar. In colonies of this age the agar film is entirely eaten away within the area of the colony, the growth made by the organisms resting on the bottom of the dish (Plate XII, fig. 3). The colony is surrounded by a zone of about a quarter of an inch in width of semi-liquefied agar, which shows as a clear ring. In older colonies, about 14 days at room temperature (16–18°), the centre of the colony begins to grow convex with a smooth surface, surrounded by a bluish area of semi-liquid gel. At four to five weeks no further liquefaction has taken place, and the colonies have become irregular with a rugose surface having a resinous appearance.

Deep colonies show a clear area of similar width. They may be irregular, oval, or angular.

AGAR STAB. Same medium as above.

A deep hollow is formed on the surface, about 3 mm. deep, in 4–5 days at 25° C. After a month or more the whole surface is liquefied, the liquefaction extending down the sides against the glass and the unliquefied gel having a convex surface.

From a young inoculum a hollow is formed within 24 hours.

GELATINE STAB. Very slight surface growth after one month; the streak then shows a beaded line. There is no liquefaction. The beading is less pronounced if the stab is made from a young inoculum.

STARCH AGAR. Of the same composition as the above agar, with the addition of 0·1 per cent. of Lintner's soluble starch. The agar is poured into sterile petri dishes and allowed to harden. When cool, the surface is streaked with the inoculum, and incubated at 25° C. In 48 hours or less a groove appears along the surface and, as in the case of the agar slope, the agar is consumed. Diastatic action is tested for by flooding the plate with a saturated solution of iodine in 50 per cent. alcohol.

3 days	7 days
Diastatic action pronounced	Stronger, 2 cm. diam.

With the most viable inoculum liquefaction begins in 18 hours.

NUTRIENT BROTH. Peptone (0·5 per cent.), Lemco (0·3 per cent.). No scum or sediment. Slightly cloudy liquid.

CARBOHYDRATE BROTH. Composed of 1 per cent. peptone and 1 per cent. of each carbohydrate as under, incubated for seven days at 25° C. Duplicate test tube cultures were inoculated in each case, and tested with five drops of neutral litmus solution.

Action on Carbohydrates.

Carbohydrate	Growth	Acid in
Dextrose	Slight	Both
Sucrose	Slight	Neither
Lactose	Slight	Both
Maltose	Slight	Both
Glycerine	Nil	Neither

NITRATE BROTH. The development of acidity, where present, was slight.

Peptone (1 %), KNO_3 (0·1 %)	No growth.
Nutrient broth with KNO_3 (0·1 %) ...	No nitrite at 7 days. Nitrite at 14 days.

CELLULOSE MEDIA. Filter paper medium, composed of a strip of filter paper half immersed in mineral salts solution of the following composition.

KNO_3	0·1 %	NaCl ...	0·01 %
K_2HPO_4	0·1	$CaCl_2$ ...	0·01
$MgSO_4 . 7H_2O$ ...	0·02	$FeCl_3$ ...	0·002

The paper undergoes rapid disintegration. After two or three days incubation at 25° C. fibres of the paper are thrown down from the level at which the paper is being destroyed. The paper then breaks in two and the two edges show very ragged outlines, taking on a pulpy consistency (Plate XII, fig. 1). After a month or more in this medium the paper becomes a pulpy mass at the bottom of the tube, but large areas of the paper appear not to have been attacked.

The nitrate in this medium is rapidly reduced to nitrite, as is shown by testing with α-naphthylamine-sulphanilic acid. After two months the red colour given by this reagent is faint, and on testing a 4 months old culture no trace of the nitrite can be observed. The solution remains on the alkaline side of neutrality. No ammonia is produced and the whole of the nitrate is utilised.

With $(NH_4)_2SO_4$ 0·1 per cent. or $NaNO_3$ 0·2 per cent. as the source of N the organism grows equally well. Less rapid growth takes place if nitrogen is not added; the amount of organic N present in this filter paper (0·018 per cent.) apparently being available to the organism. The paper is broken through in 14 days time. A square of filter paper wholly immersed in the solution is attacked much less rapidly than where the paper is half immersed.

Cellulose agar, made according to the directions of Kellerman (7, 8). No growth occurred on plates or slopes.

Cellulose sheet agar, consisting of a sterile sheet of filter paper applied to the surface of mineral salts agar and dilute suspensions of the organisms in salts agar poured on in a thin layer (Hutchinson's method for *Sp. cytophaga*). No colonies appeared, nor was the gel liquefied.

Cellulose sheet agar, a sterile strip of filter paper applied to the surface of a mineral salts agar slope was streaked with a culture of the organism. It grew well in the liquid at the bottom but not on the surface of the paper.

METHOD OF ATTACKING FILTER PAPER. The organism apparently enters the fibres and multiplies in the central canal; in young filter paper cultures cells may be seen in the living state actively motile within the fibre. In old cultures motility has ceased and the organisms have multiplied to a considerable extent, taking on the coccoid form. These two stages are illustrated on Plate XIII, figs. 2 and 3.

PLAIN AGAR MEDIA. 1·5 per cent. agar with mineral salts as given above but with various sources of nitrogen. Slope cultures were made.

1.	$(NH_4)_2SO_4$	0·1 %	Rapid liquefaction, exceeding that on nutrient agar.
2.	,,	0·06 %	Less rapid than with 0·1 %.
3.	$NaNO_2$	0·1 %	No growth took place.
4.	$NaNO_3$	0·2 %	Liquefied, but less growth than on 2.

5. Nitrogen-free	—	Slight liquefaction.
6. Nitrogen-free Mannitol	1·0 %	Slight liquefaction.

The last medium is one on which *Azotobacter* grows well. The amount of organic nitrogen present in the agar used has been found to be as high as 0·272 %, but apparently very little of this is available to the organism.

Viability. Cultures rapidly lose viability in all the media so far tested. This is due apparently to the production of some substance toxic to the majority of cells present, for very few colonies appear on plates even from a thick suspension; this is especially true of cultures a week old or older. Viability is, however, kept by some cells for as long as seven months. On the other hand, the cellulose decomposing power has not been found to decrease after the organism has been sub-cultured for nine nonths.

Systematic Position. On account of its morphological character, coupled with its remarkable power of liquefying agar-gels, the name *Microspira agar-liquefaciens* is suggested for the new organism described. A preliminary note was read soon after its discovery to a meeting of the Pathological Society of Great Britain and Ireland held at Oxford on July 13th, 1923.

The literature gives three references to organisms having the agar-liquefying power. *Bacillus gelaticus*, obtained by Gran from sea water (3), was used in conjunction with *Clostridium Americanum* and with *Azotobacter chroococcum* by E. and H. Pringsheim (12) to supply these nitrogen fixing organisms with a source of energy through the hydrolysis of agar. Panek described in 1905 (11) an organism from sugar-beet under the name of *Bacterium betae viscosum*. In 1911 Biernacki (1) isolated from raisins a facultative anaerobe to which he gave the name *Bacterium Nenckii*. None of these organisms has apparently been tested for the power of decomposing cellulose. They are all, however, considerably different in other respects from the organism here described; we are justified therefore in considering it a hitherto undescribed species.

Part II.

THE DECOMPOSITION OF CELLULOSE BY *M. AGAR-LIQUEFACIENS* IN THE PRESENCE OF SUGARS AND RELATED COMPOUNDS.

As a result of their work on the breaking down of long straw in the presence of soluble nitrogen compounds, Hutchinson and Richards (5) have developed a method by which straw and other materials rich in cellulose can be decomposed to produce a substance closely resembling farmyard manure of high fertilising value(13). Since the supplies of natural stable manure are becoming less abundant, and since, in many arable areas, farmyard manure is scarce or unobtainable, such methods of producing an organic manure by artificial means must have a great future importance in maintaining the humus content of arable soils.

In the rotting of straw, whether in farmyard manure or in the presence of artificial nitrogen compounds, the disappearance of the cellulose is only one of the processes to be considered and is intimately bound up with the bacterial decomposition of the pentosans, *i.e.* carbohydrates composed of 5-carbon atom groups, which form a considerable proportion (about 50 per cent.) of the carbonaceous matter of the straw. Mr E. H. Richards, in discussing the question of cellulose decomposition with the present authors, suggested the possibility that this decomposition, in materials such as straw, may be modified by the presence of these other carbonaceous compounds. With this possibility in view it was decided to investigate the effect of small amounts of certain other compounds on the rate at which cellulose is decomposed in pure culture. For the purpose of this work it was considered that *Microspira agar-liquefaciens* was an organism eminently suitable, owing to the rapidity with which it attacks pure filter paper and its ability to live in the presence of sugars.

The carbohydrates and related compounds selected for these investigations were certain of those that are liable to occur in association with cellulose under natural conditions.

These were arabinose, xylose, glucose, sucrose, starch, dextrin, straw gum, lignin, mannitol. The cellulose used in the experiments was Whatman's No. 41 ash-free filter paper. The mineral salts solution had the following composition:

$NaNO_3$	2·5 gm.	$CaCl_2$	0·1 gm.
K_2HPO_4	1·0	$FeCl_3$	0·02
$MgSO_4.7H_2O$ ...	0·3	Distilled water ...	1000 c.c.
NaCl	0·1		

To determine the amount of cellulose consumed it was decided to use Schweitzer's reagent. Preliminary trials of the method of estimating cellulose by means of this reagent are tabulated below. It has been thought useful, however, to give first the main details of the preparation of the reagent, as advised by Charpentier(2).

200 gms. of $CuSO_4 . 10H_2O$ were dissolved in boiling distilled water, and a solution of ammonium chloride in sufficient quantity to satisfy

the equation below, followed by excess of a 10–20 per cent. solution of NaOH, were added.

$$CuSO_4 + 2NH_4Cl + 2NaOH = Cu(OH)_2 + 2NaCl + (NH_4)_2SO_4$$

The $Cu(OH)_2$ thus precipitated was washed free from the associated salts and then dissolved in ammonia of ·92 sp. gr., so that by the end of 24 hours nearly the whole of the $Cu(OH)_2$ was in solution.

Whatman's No. 41 filter paper was dried in the steam oven and weighed. It was then dissolved in 100 c.c. of the Schweitzer's reagent, the mixture being stirred with a glass rod, and the solution allowed to stand for one hour. The cellulose was then precipitated by adding just sufficient of a mixture of 100 c.c. concentrated HCl and 400 c.c. H_2O, and, when the blue colour had gone, precipitation was held to be complete. The precipitate was caught by filtering through a weighed filter paper, and washed with a dilute (about 10 per cent.) solution of HCl, followed by hot water until neutral, to remove any traces of $Cu^{\bullet}$ or $Cl^{\bullet}$. The filter paper containing the cellulose precipitate was then dried in the steam oven. The results of this are shown below.

Weights in grammes.

No.	Filter paper (at start)	Cellulose recovered	Loss	Average
1	0·6475	0·6470	0·0005	
2	0·6330	0·6327	0·0003	0·00046
3	0·6550	0·6544	0·0006	

A second test was carried out, with the filter paper arranged on the surface of beads, to correspond as closely as possible to the arrangement to be adopted for the inoculation experiments (see Fig. 1). The cellulose was recovered from the flask by first decanting as much of the liquid as possible; Schweitzer's reagent was then poured in, and, when the paper had dissolved, the beads were washed free of the reagent, both the reagent and the washing being added to the dilute HCl. The amounts of cellulose that were recovered are here given.

Weights in grammes.

No.	Filter paper (at start)	Cellulose recovered	Loss	Average
1	1·3410	1·3300	0·0110	
2	1·3370	1·3280	0·0090	0·0100
3	1·3450	1·3350	0·0100	

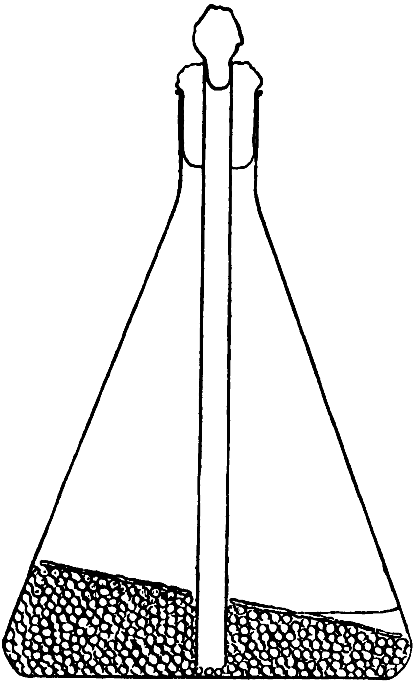

Fig. 1. Showing the orientation of the filter paper on the surface of glass beads, and the tube for inoculation.

EXPERIMENT I.

For the first experiment 500 c.c. Erlenmeyer flasks were filled to a depth of about 2 cms. with small glass beads which had been washed with HCl and then with hot water. Upon the surface of these was laid a weighed sheet of filter paper, which was folded in such a manner as to lie flat on the beads. Through the centre of this paper passed a glass tube reaching to the floor of the flask, the upper end, plugged with cotton wool, passing through the plug in the neck of the flask (Fig. 1). A solution consisting of 50 c.c. of the mineral salts solution and 50 c.c. of distilled water containing 0·1 per cent.[1] of the compound to be tested, was run into the flask *via* the glass tube. Control flasks were set up in which 50 c.c. mineral salts solution and 50 c.c. of distilled water alone were added to the beads and filter paper. Sterilisation was carried out in the autoclave at 15 lbs. pressure for 15 minutes. Each flask was inoculated *via* the tube with 1 c.c. of a suspension of a 4-day old culture of *M. agar-liquefaciens* and incubated for 10 days at 22° C. Duplicates were set up, using sand instead of beads, but this method was found to give conditions of insufficient aeration, and these cultures were not analysed in consequence.

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[1] For the reason stated in the text the concentration of lignin was much less than this.

After three or four days incubation the flasks were examined for signs of decomposition. A slight yellowing of the paper was evident, and it was becoming soft and pulpy. On gently shaking, the liquid was seen to be full of fine strands of filter paper. By the 10th day, when the whole of the paper had apparently broken down into these small fibrils, estimations of the amounts of cellulose remaining were carried out. The results of this experiment are given in Table I.

Table I.

Additional substance	Weight of paper at start (gm.)	Weight of paper recovered after 10 days (gm.)	Cellulose lost per cent.
Dextrin	0·607	0·370	39·04
Lignin	0·545	0·337	38·165
Xylose	0·580	0·360	37·93
Sucrose	0·555	0·380	31·54
Arabinose	0·585	0·426	27·18
Straw gum	0·585	0·465	20·51
Sol. starch	0·595	0·517	13·11
Control	0·587	0·548	6·64
Glucose	0·565	0·533	5·66
Mannitol	0·615	0·605	—

The amount of additional substance in each case was 0·05 per cent. of the liquid medium, save in the case of lignin. A small part only of the lignin went into solution. This substance and the straw gum were autoclaved previously for 20 minutes at 20 lbs. pressure. The straw gum went completely into solution, but only 18 mgm. of lignin dissolved in 100 c.c. H_2O. This concentration was then halved in making up the medium, giving 9 mgm. of lignin in the 100 c.c. used.

The control culture in this experiment did not show such good growth as usual because the slope of the beads was displaced and was so steep as to exclude air from a large surface of the paper. This experiment was regarded, however, merely as a preliminary one to ascertain which of the compounds used would be worthy of a more critical test.

EXPERIMENT II.

It was decided to continue with xylose, arabinose, lignin, and straw gum in a second experiment. Each substance was tested in two concentrations, 0·025 and 0·05 per cent., and duplicate flasks of each concentration were set up, the filter paper resting on the surface of glass beads as described in the first experiment, with the exception that the surface of the beads was kept horizontal. Duplicate controls, inoculated, but with no organic compound other than the cellulose, were also set up. The cultures were incubated at 22° C. for 10 days, and the amount of cellulose remaining unattacked at that time determined as described above. The results of this second experiment are shown in Table II.

Table II.

Substance added	Weight in gm. in solution	Paper at start		Paper recovered		Cellulose lost per cent.		Average loss per cent. of duplicates
		A	*B*	*A*	*B*	*A*	*B*	
Xylose	0·025	0·777	0·858	0·466	0·498	40·0	41·9	40·95
	0·05	0·737	0·715	0·435	0·429	40·9	40·0	40·45
Lignin	0·0045	0·895	0·717	0·541	0·431	39·55	39·9	39·73
	0·009	0·772	0·853	0·501	0·562	35·1	34·1	34·60
Arabinose	0·025	0·808	0·783	0·566	0·522	30·0	33·3	31·65
	0·05	0·807	0·793	0·597	0·588	26·0	25·8	25·91
Control	—	0·868	0·795	0·625	0·517	27·99	34·97	31·5
Straw gum	0·025	0·885	0·800	0·730	0·656	17·5	18·0	17·75
	0·05	0·885	0·770	0·708	0·624	20·0	18·96	19·50

It will be seen from the above table that xylose and lignin produce a very considerable increase in the amount of cellulose which is broken down in 10 days by this organism. Arabinose had no stimulating effect while straw gum caused a depression of its activity. These figures are consistent with those of the previous experiment, except in the case of the control which in the first experiment was abnormal but on this occasion gave normal growth.

The stimulating effect of xylose and lignin is of especial interest in view of the occurrence of the pentose groups in straw and of the association of lignin with cellulose in vegetable tissues. In considering the possible cause of this stimulation it was thought that it might be important that in the case of lignin the smaller concentration alone gave a significant stimulation, and in the case of arabinose the higher concentration had a depressing action. The optimum amount of such a stimulating substance may be very small, a larger quantity merely reducing the amount of cellulose destroyed by supplying an alternative source of energy. In order to ascertain whether the organism were deriving energy from the added substance the following experiment was set up.

EXPERIMENT III.

Test tube cultures were made containing 10 c.c. of the mineral salts solution, with the same concentrations of xylose, lignin, arabinose and straw gum as in the last experiment. Four tubes of each concentration contained a strip of filter paper, and four were without. Inoculations were made with a loopful of a thick suspension from the liquefied portion of an agar slope in physiological salt solution, and the cultures incubated at 25° C. Examinations were made from day to day and the following results were noted.

With filter paper.

1. Duplicate controls		Paper eaten through on the 5th day.
2. Lignin	0·0045	Same as controls.
	0·009	Paper eaten through in two tubes on the 6th day, and one on the 7th. One failed.
3. Straw gum	0·025	Paper eaten through in two tubes on the 5th day, one on the 7th, one on the 9th.
	0·05	Paper eaten through in one tube on the 5th day, one on the 6th, and two on the 9th.
4. Arabinose	0·025	Paper eaten through in one tube on the 9th day, and one on the 14th.
	0·05	Paper not attacked.
5. Xylose	0·025	Paper eaten through in one tube on the 9th day.
	0·05	Paper not attacked.

Without filter paper.

The organism grew well on straw gum, especially in the higher concentration, in 3 days. None of the other substances supported any growth.

In test tube cultures, then, none of these compounds stimulate the organism to greater activity in destroying cellulose, but, on the contrary, are inclined to retard it. Straw gum alone of these four compounds supplies it with an alternative source of energy.

EXPERIMENT IV.

In considering the stimulating action of xylose on the decomposition of cellulose it was important to ascertain whether this was due to a hastening of the initial growth of the organism and to a consequent shortening of the time before it commenced to affect the cellulose. To test this point a series of test tube cultures was set up with a wide range of concentrations of xylose, and the time taken for a visible attack of the cellulose was noted. Four tubes of each concentration were set up, the mineral salts being of the same concentration in each series, and inoculated with a drop of a thick suspension from a small separating funnel. The concentrations of xylose were in the percentages as under:

1·0	0·5	0·25
0·1	0·05	0·025
0·01	0·005	0·0025

Four tubes with no xylose were incubated as controls. Incubation was carried out at 25° C., and examinations made daily. The times were noted at which the paper was (I) showing the first signs of being attacked,

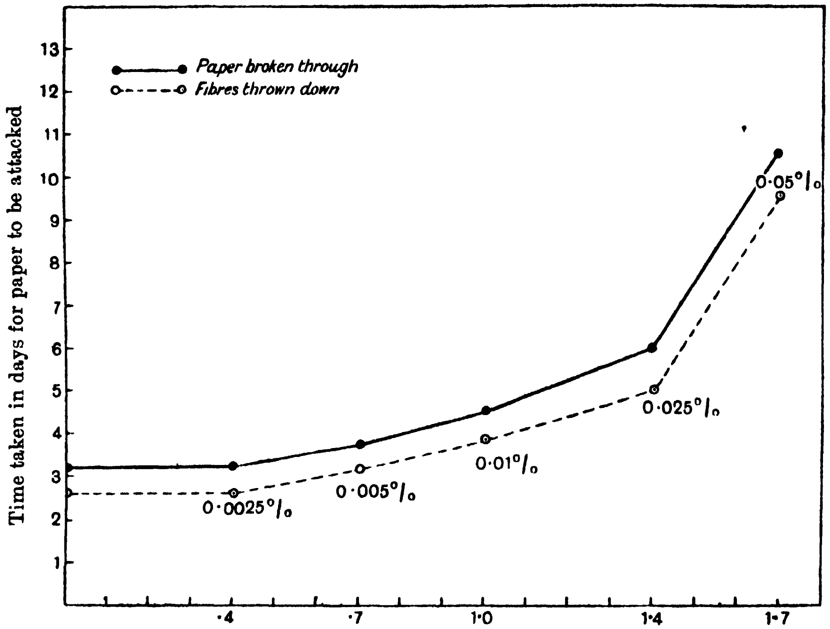

Fig. 2. The effect of varying concentrations of xylose in delaying the action on filter paper by *M. agar-liquefaciens.*

which was indicated by the throwing down of fibres into the liquid, and (II) eaten through; this latter was easily seen if the tubes were shaken gently. The appended graph shows the concentrations of the added xylose plotted against the time at which the first signs of attack and the breaking of the paper occurred (Fig. 2).

Concentrations above 0·05 per cent. are not included in the above graph, as no growth occurred in any of them. The points plotted represent the mean of four tubes, except in the case of 0·05 per cent. where only two tubes showed the required characteristic. The figures ·4 to 1·7 are the logs. of the milligrammes of xylose per 100 c.c. of culture fluid. In the control cultures the paper was broken through in the same time as in the case of the lowest concentration.

The curve obtained from this experiment shows that in test tube cultures concentration of xylose above 0·25 mg. in 10 c.c. extends the time required for the paper to be broken through at the air-liquid level, and that at no concentration tested did the xylose shorten this period. This delaying action of xylose in all but the weakest concentration is apparently not due to the carbohydrate acting as an alternative source of energy since the delaying concentration is so small that the energy supplied by it seems negligible. Moreover, Exp. III indicates that the organisms do not use xylose or lignin as a source of energy.

With regard to the mode of action of small amounts of xylose and lignin, in increasing the amount of cellulose decomposed, the view that these substances supply enough energy to hasten the initial growth of the organisms is rendered improbable by the considerations mentioned above, and also by the fact that, at any rate in conditions of test tube cultures, the weakest concentrations of xylose tested did not shorten the period taken by the organism to commence attacking the cellulose.

The fact that xylose and lignin may be stimulating or harmful to cellulose decomposition according to their concentration seems to be of great interest in view of the varying ratios of cellulose, lignin and pentosans met with in natural vegetable products. It seems possible that a continuation of this line of investigation may help to explain the varying rates at which different classes of plant residues are decomposed.

SUMMARY.

(1) A new micro-organism is described that possesses the power of decomposing cellulose (filter-paper) and agar-agar with great rapidity. It is able to utilise either of these substances as the sole source of energy for its growth. No loss of the power to decompose cellulose or agar has been observed after nine months cultivation in the laboratory.

(2) On account of its morphology and physiological characters the organism has received the name *Microspira agar-liquefaciens*.

(3) Pure culture experiments with this organism show that under conditions of adequate aeration it will decompose filter paper to a greater extent in the presence of small quantities of certain other carbohydrates and related substances. The stimulating effect of xylose and lignin is of especial interest owing to their association with cellulose in straw.

We are much indebted to Mr H. G. Thornton for his valued help and criticism during this work; to Mr S. du Toit for supplies of xylose and lignin, and for the estimation of lignin in solution; and to Mr G. C. Sawyer for estimations of the amount of nitrogen in filter paper and agar.

REFERENCES.

(1) Biernacki (1911). *Cent. Bakt. Par.* II, xxix, 166–9.

(2) Charpentier (1921). *Inaug. Diss. Tavastehus* (Finland).

(3) Gran (1902). *Bergens Museum Aarbog*, No. 2.

(4) Hutchinson and Clayton (1919). *Journ. Agri. Sci.* ix, 143–73.

(5) Hutchinson and Richards (1921). *Journ. Ministry of Agriculture,* xxviii, 398–411.

(6) van Iterson (1904). *Cent. Bakt. Par.* II, xi, 689–98.

(7) Kellerman and McBeth (1912). *Ibid.* II, xxxiv, 485–94.

(8) Kellerman, McBeth, Scales and Smith (1913–4). *Ibid.* II. xxxix, 502–22.

(9) McBeth and Scales (1913). *U.S. Dept. Agri. Bur. Plant Ind. Bull.* 266.

(10) Omeliansky and Solunskoff (1915). *Arch. d. Sci. biol. publ. par l'Instit. impér. d. méd. expér. à Pétrograd,* T. 18, 459–82, abstr. in *Cent. Bakt. Par.* II (1919), xlix, 473–4.

(11) Panek (1905). *Bull. de l'Acad. Sci. de Cracovie; cl. d. sci. natur.* Janvier.

(12) Pringsheim, E. and H. (1910). *Cent. Bakt. Par.* II, xxvi, 227–31.

(13) Rothamsted Exp. Station. *Report,* 1921–2, 92.

(14) Truffaut and Bezssonoff (1920). *Comptes Rendus,* 171, 1089.

EXPLANATION OF PLATES XII AND XIII.

PLATE XII.

Cultures of Microspira agar-liquefaciens.

Fig. 1. Test tube culture three days old showing filter paper broken through. (Nat. size.)

Fig. 2. Nutrient agar slopes. On the left a sterile slope; on the right a streak culture of *M. agar-liquefaciens* showing increase in liquid at the bottom. (Nat. size.)

Fig. 3. Five days old agar plate showing depressed colonies and refraction of light from semi-liquefied agar. (× ⅔.)

PLATE XIII.

Camera lucida drawings of M. agar-liquefaciens.

Fig. 1. *M. agar-liquefaciens* showing flagella. Stained by P. H. H. Gray's (Rothamsted) method. (× 2500.)

Fig. 2. Fibre of filter paper (three days old culture) showing organisms inside the central canal. Unstained. (× 1200.)

Fig. 3. Fibre of filter paper, one month old, showing organisms in the coccoid stage inside the central canal. Stained *intra vitam* with carbol fuchsin. (× 1200.)

(*Received March 26th,* 1924.)

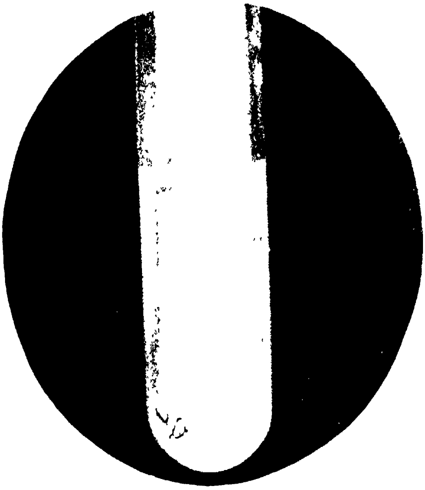

Fig. 1.

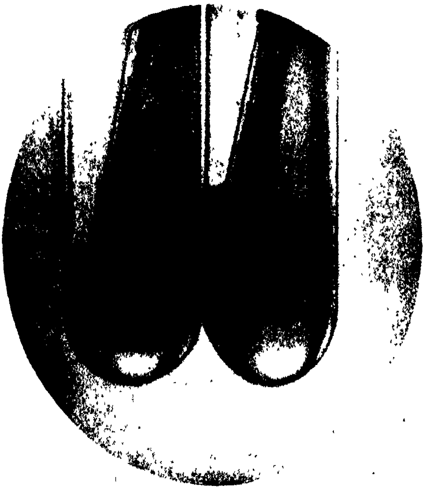

Fig. 2.

Fig. 3.

GRAY & CHALMERS.—On the Stimulating Action of certain Organic Compounds. (pp. 324–338)

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PLATE XIII

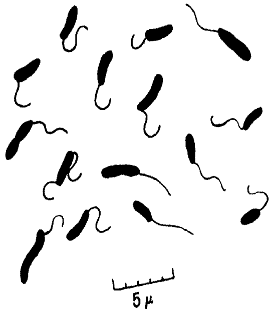

Fig. 1.

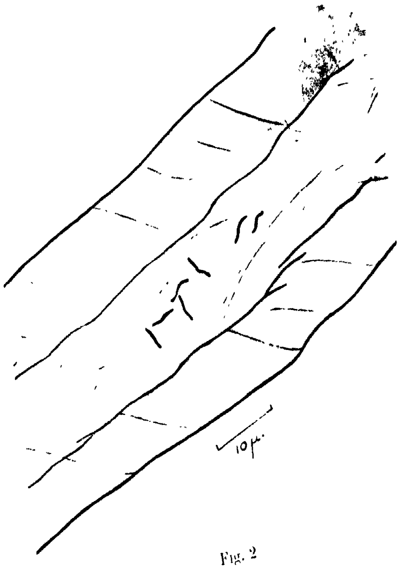

Fig. 2

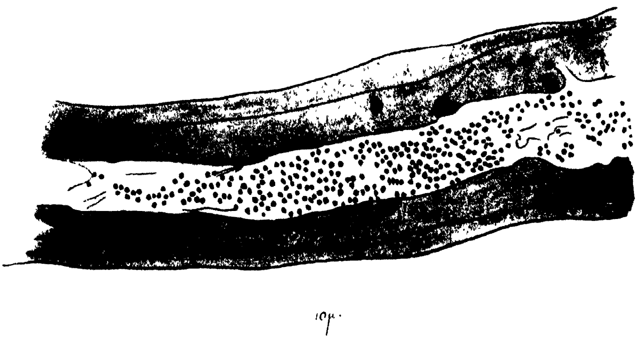

Fig. 3.

GRAY & CHALMERS.—On the Stimulating Action of certain Organic Compounds. (pp. 324—338.)

OBSERVATIONS ON THE LIFE HISTORY OF TAPEWORMS OF THE GENUS *MONIEZIA*

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(With Plate XIV.)

It is a remarkable fact, that whilst numerous species of tapeworms belonging to the genera *Moniezia* (Blanchard, 1891); *Thysanosoma* (Diesing 1834); and *Stilesia* (Railliet 1893), are common parasites of herbivorous animals, in no case has the intermediate host of the parasite been discovered.

The present paper records an attempt to elucidate the life histories of those species of *Moniezia* commonly parasitic upon lambs in the Aberystwyth district, and although the conclusions reached are largely negative, it is put forward in the hope that some portion of the ground may be cleared for other investigators.

Numerous investigators have embarked upon the problem, the results of their observations having recently been dealt with by Flattely (1), who gives a most comprehensive historical account of their work, together with an account of his own investigations on the subject, and a full bibliography, to which the reader is referred.

Although some authorities support the theory of direct infection without the intervention of an intermediate host, numerous attempts to substantiate the theory have been fruitless, and the observations here described were made on the assumption that the life histories of *Moniezia* spp. are in accordance with those of the great majority of Cestodes—presupposing, therefore, the existence of an intermediate host.

The outstanding feature of the infection of lambs with *Moniezia* spp. is the very early age at which infection occurs. On March 17th, 1922, at a farm in the Aberystwyth district, mature proglottides were found in lamb droppings, just over five weeks from the commencement of the lambing season. Since a lamb of five weeks old is not weaned, and does not nibble pasture in more than a very casual manner, the fact that heavy infections occur at such an early age has led many investigators to support the theory that infection takes place from the mother, either *via* the maternal milk, or from external parasites living on the surface of the udder, and taken in by the lamb during sucking operations, or from some larval stage of the tapeworm which, present in the herbage, becomes transferred to the udder when the mother ewe lies down. In the absence of evidence to the contrary, the theory that infection is contracted from the mother ewes receives support from Flattely (1); it is considered, however, that the observations here recorded render the theory untenable.

PERIOD OF THE YEAR DURING WHICH INFECTION OCCURS.

The majority of infections take place in the spring, when the lambs are a few weeks old, and it has been hitherto supposed that infection is confined to that period. It has now been established, however, that infection is not confined to the spring. On October 12th, 1921, fifteen tapeworms were collected from lambs which had been born the previous spring. Seven of these tapeworms were mature, varying in length from 94 cm. to 138 cm.; they had evidently shed many proglottides, and were the outcome of an infection which had taken place the previous spring. The remaining eight tapeworms were complete, that is, the original end segments possessing the terminal excretory pore were present, showing that no ripe segments had been shed. The most posterior segments, although possessing well-developed uteri, were by no means fully mature. These worms were evidently the result of an infection which took place at a much later date than the one which gave rise to the seven worms referred to above. Since a tapeworm collected on March 31st, 1922, from a lamb four months old, whilst possessing the terminal segment with excretory pore, was more fully mature than these eight, it is probable that the infection to which the latter were due took place at least subsequent to July, 1921.

DURATION OF THE PERIOD OF INFECTION.

Since it is not possible to examine lambs in any number, for tapeworm infection, until they are slaughtered, usually at about four months old, and since the rate of growth of the tapeworms is not known, it is not possible to fix accurately the earliest date at which infection may occur. As stated above, however, mature proglottides were being shed on March 17th, 1922, and since the longest worm complete with terminal segment which has been collected measured 240 cm., it is clear that for mature proglottides to be shed on March 17th, infection must have taken place some little time prior to that date.

On May 18th, 1922, two hundred and thirty tapeworms were taken from a single lamb aged about four months. Care was taken when enumerating them, to count only those possessing scolices. Eight of these tapeworms were broken, the remaining two hundred and twenty-two were unbroken, and had shed no segments, each possessing the original terminal segment. These tapeworms varied in length from 123 cm. to 3 mm., the variation being gradual as shown by the following table. Measurements for the sake of briefness have been reduced to the nearest ·5 cm.

Length in cms.	No.	Length in cms.	No.
123	1	11	3
106	1	10·5	2
38	1	9·5	1
30·5	1	8	7
28·5	1	7·5	6
27	1	7	3
26·5	1	6	11
25·5	2	5·5	2
24·5	1	5	11
24	1	4·5	5
23·5	1	4	12
20	2	3·5	12
19	1	3	22
17·5	1	2·5	21
16	3	—	—
15·5	1	2	20
15	1	1·5	23
14·5	1	—	—
14	1	1	22
12·5	2	·5	12
12	2	—	—

Assuming all the tapeworms to have grown at the same rate, it would appear that infection had been continuous for some weeks up to the middle of May, and that the later infections were the heaviest.

The fact that all the tapeworms resulting from larval forms ingested do not necessarily persist has not been lost sight of, and is dealt with below. This table should therefore be read in conjunction with the discussion as to the probable period of maximum infection, which follows.

It was found impossible to obtain reliable statistical information as to the exact period of maximum infection, for the following reasons:

A. It was not possible to examine the intestines of every lamb slaughtered.

B. During the period when the lambs slaughtered were found to be most heavily infected, the large number of tapeworms occurring in each lamb (230 being the greatest number found in any one lamb) made it impossible to count and measure all the tapeworms collected, before post-mortem decay set in. All disentanglement of worms—no light undertaking—had to be done before fixing, as the worms after fixing were too brittle to permit much handling. All measurements had also to be made before fixing, as considerable expansion or contraction may occur during the fixing process, making post-fixation measurements not strictly comparable one with the other.

Reliable figures indicating the intensity of infection at different dates are thus not available, but it can be stated with confidence that general observations show infection to commence about the beginning of March (see above), that the rate of infection increases until the end of April or the beginning of May, when it is at its maximum. The rate decreases after that date, and infection may occur at least until the beginning of August (see above).

These conclusions are arrived at by observations, not of the periods when most tapeworms were found in the lambs, but of the periods when most young tapeworms indicating the occurrence of a recent infection were found. This procedure was necessary owing to the fact that the older the lambs get, the smaller the number of tapeworms they are found to harbour. Two possibilities suggest themselves in explanation of this fact, firstly, that it is probably a physical impossibility for a lamb to harbour a large number (say 200) of fully grown worms, and that some are forced out of the intestine by the growth of those more securely attached to the intestinal wall; and secondly, that the probable change in the digestive juices, consequent to the change from a milk diet to a pasture diet, creates an environment less favourable both to the development of any larval forms which may be ingested, and to the continued presence of those tapeworms already harboured.

LIABILITY OF SHEEP TO INFECTION.

It has hitherto been supposed that lambs only are liable to infection by *Moniezia* spp., and that any tapeworms found in sheep have persisted from an infection which occurred when the sheep were lambs.

On March 16th, 1922, a small *M. translucida* was collected from a three year old sheep. Although the scolex was unfortunately not present, from the fact that the most anterior segments showed no trace of the rudiments of reproductive organs, and the original terminal segment, complete with excretory pore, was present, it was clear that the whole tapeworm could not have exceeded 10 cm. in length. It was, therefore, the result of a very recent infection.

On April 4th, 1922, a young tapeworm, 7 mm. long, was taken from a yearling sheep born the previous spring. The worm showed no trace of segmentation and was obviously the outcome of a very recent infection.

These observations have since been confirmed by other workers at Aberystwyth, who have also taken young tapeworms from yearlings and from adult sheep. It seems probable that infection both of sheep and of lambs may occur at any period of the year, but that the digestive juices consequent to a herbivorous diet are, as mentioned above, not favourable to development of the larval form.

The observations recorded above show that infection is not confined to the sucking period, and may take place long after the animal concerned has been weaned. They appear, therefore, to render the following theories untenable:

A. *That infection occurs* via *the maternal milk, or from larval forms existing externally upon the maternal udder.*

B. *That a milk diet is essential to development of the larval form.* The fact that lambs are infected to an enormously greater extent than are sheep, has given rise to the theory that a milk diet is essential for the development of the larval stage. These observations show that a milk diet, while it may be favourable to such development, is not essential.

DISTRIBUTION OF THE GENUS.

In ascertaining the distribution of *Moniezia*, farms have been regarded as infected, only when tapeworms have been collected from lambs bred on those farms, and reared there up to the time of slaughtering. Owing to the fact that lowland farmers are in the habit of buying in sheep from the uplands in the Autumn, the presence of *Moniezia* in a sheep is not necessarily an indication that the farm from which it was sold for slaughtering is infected.

In all, thirty-four farms were found to be infected. It was hoped that some correlation might be established between the physical characteristics of a farm and the presence or absence of *Moniezia* infection, and thus, that a clue would be obtained to the life history of the parasite. Unfortunately observations have not revealed the existence of any such correlation; farms of all types were found to be infected, and no factor common to all, and giving a clue to the life history, could be discovered.

Infected farms were found from sea level to 600 feet; wet marshy farms, dry hillside farms; farms on light land and farms on heavy land, were all infected, whilst the characteristic vegetation varied with altitude, type of soil and moisture content. One instance in particular may be mentioned. A certain farm near Aberystwyth is divided into two clearly defined portions, one consisting of dry hillside pasture land, running up to about 200 feet, the other of low lying water-logged land, merging into a marsh, practically at sea level. The latter low lying portion was sublet by the farmer, and on it the sub-tenant raised a crop of lambs. On the dry hillside pastures the farmer also raised a crop of lambs. At no time did the two flocks intermingle, but were kept entirely separate. Lambs from both flocks were found to be equally heavily infected with *Moniezia.*

As far as present observations show, therefore, altitude to 600 feet, type of soil, water content of soil or type of vegetation, do not appear to be factors influencing the infection of lambs with *Moniezia.*

FAUNA OF INFECTED FARMS.

An examination of the fauna of infected farms during the infective period showed that the variety of possible intermediate hosts present on pastures at, and immediately after, lambing time, when infection starts, is very small, and is practically limited to field slugs, dung beetles and dung-living Staphylinids and Mites, all clearly living under the best possible conditions for the ingestion of tapeworm eggs.

Since, as described above, it is possible for a lamb to harbour very large numbers of tapeworms, three possibilities must be considered:

A. The intermediate host may be extremely common on the pastures at lambing time.

B. The intermediate host may not be common, but may harbour very numerous tapeworm larvae.

C. The intermediate host may neither be common, nor harbour large numbers of larvae, but the larval form may be of a multiple type, *i.e.* either a Coenurus or an Echinococcus.

It is, of course, possible for all three conditions to be co-existent, but further consideration shows that the first at all events must be held to exist. If this were not so, although individual lambs might be found to be heavily infected by the existence of conditions two and (or) three, one would expect infection to be patchy, that is, that a small proportion of lambs only in any flock would be found to be heavily infected, and that the remaining lambs would not be infected at all. This, however, is not so; when a flock is infected with *Moniezia,* practically all the lambs are infected, and the degree of infection of individual lambs varies from very heavy to very light.

One is thus forced to the conclusion that the intermediate host is very common, and this conclusion has importance in that, excluding the external parasites of sheep and lambs, it narrows down the possible intermediate hosts to members of the groups detailed above.

With regard to the possible occurrence of condition "B," that is, that the intermediate host may harbour large numbers of the larval forms, the supposition that this is so has its attractions. It would help to explain, for instance, why the percentage of lambs infected is very high, having regard to the fact that at the time of maximum infection, the lambs do very little nibbling of pasture. Thus, if the intermediate hosts were common, and also harboured large numbers of larvae, a lamb, although little nibbling were done, might easily ingest a single intermediate host, and so become heavily infected at once. It must be remembered, however, that where a lamb is heavily infected, the worms are not found to be all of the same age, as one would expect, if infection occurred through the ingestion of a single intermediate host. On the contrary, the worms are all of different ages, as shown by the figures tabulated above, indicating, therefore, the ingestion of numerous intermediate hosts.

The existence of the third condition, however, does not seem probable. It seems extremely likely that the intermediate host, if one exists, will prove to be some small invertebrate, and that the larval form will follow the usual rule in such cases, and will be of the Plerocercoid or Cysticercoid type. Having then been forced to the conclusion that the intermediate host must be extremely common, attempts were made to produce infection of possible hosts by feeding them with mature proglottides. Field slugs and dung beetles being the commonest of the pasture living forms at lambing time, attention was confined to these two groups, and in the Spring and Summer of 1922, the following experiments were carried out.

As a preliminary to the feeding experiments, the whole intestinal contents of two lambs, from each of which very young tapeworms of from 2 mm. to 5 mm. in length had been taken, were examined in the hope that since infection had taken place very shortly before, some recognisable relics of the intermediate hosts might be discovered. The whole of the intestinal tract was washed out with water, the washings sedimented several times in glass cylinders, and the whole of the solid residue carefully examined. The result unfortunately was entirely negative.

FEEDING EXPERIMENTS.

With Arion ater. Six slugs were fed for five days on ripe proglottides, and afterwards killed, one per day, and examined.

With Agriolimax agrestis. Two batches of slugs, one of fifteen, and the other of seventeen, were fed with mature proglottides for five days, and were then killed and examined at the rate of three per day.

Both species of slugs devoured the proglottides voraciously.

Two methods of examination were adopted:

A. *By dissection.* The slugs were carefully dissected under the binocular microscope, and whilst the whole of the animal was examined, particular attention was paid to the pulmonary and body cavities, and to the digestive system, the contents of the alimentary canal being removed and examined separately.

B. *By serial sections.* One specimen of *A. ater* and three of *A. agrestis* were stained with borax carmine, and examined by means of serial sections.

By neither method of examination was the slightest evidence of any development of the tapeworm eggs obtained. An examination of the faeces of the slugs showed that the majority of the eggs passed through the alimentary canal unchanged, whilst a few showed traces of what were apparently disintegration changes.

With dung beetles. The following species of dung beetles are all common in sheep dung in the Aberystwyth district, and were subjected to feeding experiments:

Onthophagus nuchicornis	5	specimens fed
Aphodius fossor	16	,,
A. fimetarius	11	,,
A. merdarius	9	,,
A. haemorrhoidalis	19	,,

The beetles were fed with mature proglottides, which they eat with avidity, for from three to five days and were killed and examined at daily intervals up to three weeks. Examination was by means of dissection under the binocular microscope, whilst the contents of the alimentary canal were removed and examined separately. Results were entirely negative.

EXPERIMENTS WITH SHEEP KEDS.

The Sheep Ked, *Melophagus ovinus*, was next considered as a possible intermediate host. Since sheep must often lie down on dung containing mature proglottides, it is extremely likely that the wool at times must harbour large numbers of *Moniezia* eggs, and therefore that the Ked almost certainly must often ingest them. Keds were therefore collected at the slaughterhouse from the fleeces of lambs found to be infected with tapeworms, and were carefully examined, by dissection. Thirty-two Keds were thus dealt with, with entirely negative results. Attempts to keep Keds alive in captivity, and to feed them with *Moniezia* eggs, failed.

In all feeding experiments care was taken to provide mature proglottides from as many species as possible.

DETAILS OF SPECIES COLLECTED.

The following five species of *Moniezia* have been collected at the slaughter house, Aberystwyth.

Alba Group. M. alba (Perroncito, 1879).
Planissima Group. M. benedeni (Moniez, 1879).
M. pellucida Blei, 1921
M. translucida Jenkins, 1923.
Expansa Group. M. expansa (Rudolphi, 1810).

In addition, three other forms have been collected, whose specific position has not been determined. *M. trigonophora* (Stiles and Hassall, 1893), taken by Flattely at Aberystwyth(1), has not been taken by the writer.

It should be added that Flattely does not wish to attach too much importance to the occurrence of *M. trigonophora* in Wales, on account of the fact that he has not found it elsewhere, and that the triangular arrangement of the testicles, one of the diagnostic features, is occasionally shown by *M. expansa.*

A study of the genus *Moniezia* convinces one that a revision of the genus is extremely desirable. As has already been pointed out by the writer(2), forms are at present given specific designation on descriptions far too scanty to justify their inclusion in the genus even, the descriptions often being confined to the most meagre details of externals. Identifications based on observations of externals are useless unless they are supplemented by details of the internal anatomy; not only may two members of the same species differ so much externally as to be mistaken for different species, but two members of different species may resemble each other so much as to be mistaken for members of the same species.

The enormous number of tapeworms occurring during the period of maximum infection, and the fact that identification must be based on both external and internal anatomy, makes it impossible to be sure that all species occurring in the district have been identified and also to obtain reliable statistics of the frequency of occurrence of each species. The writer is of the opinion that further observations would reveal the occurrence of other species in addition to the ones already identified.

With regard to the number of species occurring in any one individual, the usual condition is that the tapeworms found in one lamb are of one species. The rule, however, is not invariable, as *M. expansa* and *M. translucida* have been found in one individual, as have *M. expansa* and *M. alba*.

SUMMARY.

The period during which lambs may become infected with *Moniezia* spp. is not confined to the Spring, but may extend at least until August.

Sheep as well as lambs may become infected.

Neither the distribution of the genus nor the fauna of infected farms has been of guidance in discovering the life history.

Examination of the intestinal contents of lambs which had become heavily infected shortly before death gave negative results.

Attempts to produce infection of possible intermediate hosts by feeding with mature proglottides gave negative results.

Five species of *Moniezia* have been collected at Aberystwyth, and there is every reason to suspect the occurrence of further species.

REFERENCES.

(1) **Flattely, F. W.** Considerations on the Life History of Tapeworms of the Genus *Moniezia*. *Parasitology*, xiv, Nos. 3 and 4, December, 1922.

(2) **Jenkins, J. R. W.** On a New Species of *Moniezia* from the Sheep, *Ovis aries*. *Annals of Applied Biology*, x, No. 2, July, 1923.

EXPLANATION OF PLATE XIV.

Fig. 1. Young tapeworm collected on April 4th, 1922, from a yearling sheep born the previous spring. Segmentation has not commenced.

Fig. 2. Terminal segments of a young specimen of *M. translucida* collected on March 16th, 1922, from a three year old sheep. The genital organs are still in *anlage*, and the terminal excretory pore is present.

Fig. 3. Terminal segments of a young specimen of *M. translucida* collected on March 31st, 1922, from a lamb four months old. This specimen is clearly much more mature than that illustrated by Fig. 2. Fertilisation has been effected as shown by the swollen receptacula, whilst the uteri have spread considerably.

(*Received April 3rd*, 1924.)

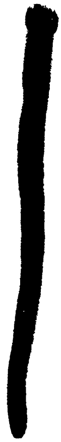

Fig. 1.

Fig. 2.

Fig. 3.

JENKINS.—OBSERVATIONS ON THE LIFE HISTORY OF TAPEWORMS OF THE GENUS *MONIEZIA*. (pp. 339—348.)

THE SOIL POPULATION. AN INVESTIGATION OF THE BIOLOGY OF THE SOIL IN CERTAIN DISTRICTS OF ABERYSTWYTH

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(With 7 Text-figures.)

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1. INTRODUCTION.

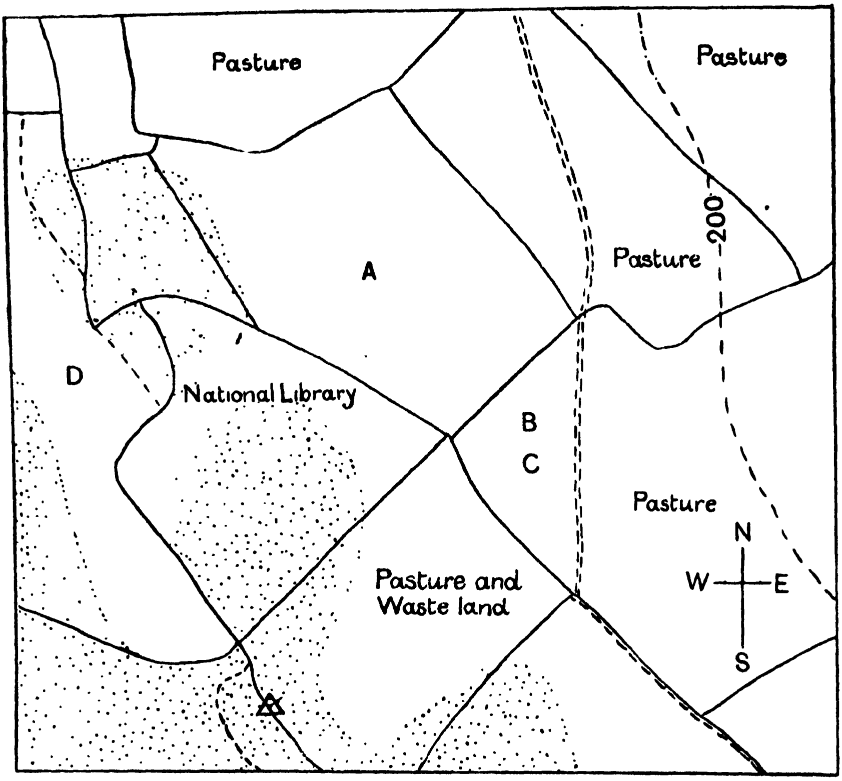
THE following paper gives an account of a systematic investigation of the soil from certain areas at Aberystwyth, extending over a period of more than two years.

The investigation is mainly concerned with a quantitative seasonal examination of the soil fauna, together with some reference to the vegetation, humus content, physical nature of the soil and to the atmospheric conditions. An attempt has been made to include all organisms in the soil which are visible to the naked eye or by using a hand lens. The observations recorded offer an interesting comparison with those obtained by Cameron (4, 5), Morris (19, 20) and Buckle (3).

A preliminary study of the soil fungi has been carried out, the results of which, however, are not included in this paper, except in so far as they are directly concerned with the soil fauna.

The work has been carried out on land belonging to the College plant breeding station, just north of Aberystwyth, about two-thirds of the distance up the valley side of the Rheidol river, and about three-quarters of a mile from the coast.

The particular areas examined are within a short distance of each other, and were chosen as appearing to be typical of their kind in the district. They agree in that they are formed of soil essentially the same in physical, chemical and geological character. They consist of: (*a*) PERMANENT PASTURE which forms the main topic of the paper; (*b*) GRASSLAND similar in nature to the pasture, save that it is ungrazed; (*c*) land from which the turf had been entirely removed a short time before the investigation and (*d*) land under cultivation.



A. Pasture field. B. Ungrazed grassland. C. Land recently ploughed. D. Cultivated land. ::: Terannon, Llandovery.

Fig. 1.

The land of the Aberystwyth district consists mainly of shales and grits from the Ordovician and Silurian systems, overlain in many parts by boulder clay or alluvial deposits. The upper layers of the areas investigated consist of the Terannon formation of the Llandovery (Silurian) overlain by boulder clay (Fig. 1), so that the soil is probably formed by an intermingling of these. The majority of stones present in the soils are either of the boulder clay type or fragments of laminae from the shales. The soil is fertile with a moderate amount of humus to a depth of at least nine inches and shows a good growth of vegetation.

The physical characters of the soil allow free gaseous diffusion among the particles, thus affording favourable conditions for life within the soil. Aeration is further increased by the long and spreading tap root systems of certain plants which aid in opening up the soil. Hole (13) in his paper on "Soil Aeration" states that in ordinary arable or pasture land, the air differs little in its composition from atmospheric air, the amount of oxygen being somewhat less and the carbon-dioxide a little more. It is probable that the degree of aeration of the soil is one of the main factors regulating the vertical distribution of the macroscopic fauna; aeration decreases with depth. When the soil is loosened by ploughing or hoeing aeration is efficient for a greater depth with a correspondingly deeper distribution of the fauna. The family *Achorutidae* of the *Collembola* illustrate this particularly well, the numbers obtained from the 6 to 9 inch layer almost equalling those from the 3 to 6 inch or the surface 3 inch layer. This is in marked contrast with grasslands, where, in the investigation, macroscopic organisms were to a marked degree most numerous in the surface 3 inches, the numbers becoming steadily reduced in the lower layers, specimens occurring but rarely below 12 inches.

Again, a number of soil organisms are root feeders and would thus, particularly in grassland, be concentrated in the upper layers of soil.

Another main factor influencing vertical distribution is the occurrence of humus.

Ploughing and hoeing have a destructive effect on many forms, and this fact was most clearly demonstrated by records of certain samples where the numbers of the *Collembola* reached 169 in a sample from an undisturbed portion of arable land, against 25 from a part hoed a few days before examination. Apart from direct injury to larvae and the larger forms, the disturbance of the soil naturally results in increased evaporation of moisture from the surface, causing temporary drought. As stated by certain investigators, this has a particularly detrimental effect on *Collembola*.

Excessive ground water appears to be the greatest mechanical enemy of the soil fauna, but in the areas investigated there is little danger of water-logging owing to the geological formation of the land and the physical nature of the soil.

The average and equable conditions to which the areas are subjected are indicated by the typically mesophytic vegetation.

Fungal attack has been small, and, so far as observed, restricted to injured organisms. Also, there has not been any excessive occurrence of the recognised harmful types of fauna; such were present but were only found occasionally, either singly or in small numbers.

2. METHOD FOR INVESTIGATION OF THE SOIL FAUNA.

(*a*) COLLECTING. Samples of a 9-inch cube in size were taken and were collected in three layers, each 9 × 9 × 3 inches, to obtain a quantitative estimation of the soil fauna at different depths. Samples were taken several feet apart from each other.

(*b*) EXAMINATION OF THE SAMPLES. After experiment, the best method of procedure has been found to be as follows:

(i) A preliminary sorting of the soil particles over brown paper and examination for organisms.

(ii) After this preliminary sifting, a small portion of the soil is again taken and placed in a sieve of about ·75 mm. mesh and a slow stream of water allowed to run in. The sieve is at the same time shaken, to allow the fine silt to pass through the mesh, and this is caught in a wide shallow dish. When no more sediment passes through both residue and filtrate are examined.

Although examined time after time with a high power hand lens, no organism has ever been found in the filtrate, so it may be accepted that no macroscopic forms pass through the sieve. The smallest organisms taken into consideration in this investigation appear as tiny specks to the naked eye. After the preliminary experiments the filtrate was neglected.

The residue is carefully washed out from the sieve into a wide shallow dish and water added until the dish is half full; the residue is stirred gently, thus releasing any organisms between the particles, which then float up to the surface of the water. The latter is examined with a hand lens or a binocular microscope. Several organisms which are difficult to detect by the first sorting are revealed by this method.

The water is poured off and the process repeated with fresh water until nothing further is found; usually once is sufficient.

Finally, the actual solid residue is examined, though at this stage there is rarely anything present.

It is necessary to go through the preliminary sorting, as the larger forms are very liable to be bruised during sieving, though the smaller ones escape uninjured. The larger pupae especially were in some cases so damaged that it was impossible to rear them for purposes of identification.

3. PERMANENT PASTURE LAND.

(i) ECOLOGY. The chief area to be discussed is a field of permanent pasture, about 2060 square feet in area, and approximately 200 feet above sea level. It is surrounded by other pasture fields to the S.E., E. and N., one of which was an ungrazed area for several years, but since January 1921 has been used for agricultural experiments with wheat and grasses. A strip of waste land lies between the area in question and the buildings of the National Library.

Two sides of the pasture field are bounded by thin hawthorn and blackthorn hedges, on vertical banks about 4 feet in height. These hedges are clean with little variety in vegetation beneath them. The remaining sides of the field are marked by wire.

A stream runs across an adjoining field, but apparently has little effect on the fauna of the neighbouring land.

The field has a fairly uniform surface, evenly covered by the vegetation. The main slope of the ground is to the N.E., a dip of about 2°; there is also a scarcely perceptible one to the N. Owing to this slope, drainage leads away from the area.

A small amount of shelter from certain winds is afforded by rising ground and the National Library.

Grazing by horses or cows is fairly regular during Autumn, Winter and early Spring; hens, crows, pigeons and smaller birds are frequent visitors. Thus, the field may be said to be under conditions of natural manuring. For the remaining months the grass is allowed to grow for hay.

The vegetation is essentially mesophytic and is that of an ordinary pasture field in which the species are not numerous. The dominant plants are *Cynosurus* and *Poa*, which form a compact turf layer with here and there small patches of the coarser grasses, notably *Dactylis glomerata*; a small, low-growing moss, *Eurhynchium* sp., is very common everywhere, and is chiefly abundant on the clover patches. Weeds are scattered unevenly over the field.

The following list gives a relative scale of frequency for the main plants belonging to this area; the number 10 denoting the dominant forms. *Cynosurus cristatus* 10; *Poa annua* 10; *P. pratensis* 10; *Eurhynchium* sp. 10; *Ranunculus bulbosus* 8; *Cynosurus echinatus* 7; *Poa trivialis* 7; *Dactylis glomerata* 7; *Trifolium repens* 7; *Ranunculus repens* 5; *Cerastium vulgatum* 5; *Urtica dioica* 5; *Rumex crispus* 4; *Carduus pratensis* 4; *Veronica Chamaedrys* 4; *Bellis perennis* 4; *Trifolium pratense* 3; *Chrysanthemum leucanthemum* 3; *Euphrasia officinalis* 3; *Trifolium incarnatum* 2; *Rumex acetosa* 2; *R. acetosella* 2; *Ranunculus acris* 2; *Taraxacum-dens-leonis* 2; *Plantago lanceolata* 1.

High S.W. winds are very characteristic of the district, not infrequently in the form of gales; these usually result in increased evaporation of moisture from the soil, although being typically "wet" winds, there is less danger from this than might be expected. Probably a certain amount of sea spray is carried but not enough to cause any marked difference in the type of animal or vegetable life.

Precipitation is fairly high, mainly brought by these S.W. winds.

The *water content* of the soil plays a great part both in animal and vegetable life; success and healthy existence depend largely on the condition that water shall be present in an available form(4). It has been found that hygroscopic water is the most essential for plants and capillary water for animals; excess of gravitation water blocks the air spaces and causes the death of both plants and animals. Owing partly to the porous and open nature of the soil in the areas under investigation and the shaley substratum, excess of water drains away to a great extent. On the other hand, drought prolonged for more than a very short time is usually fatal; for example, *Collembola* almost entirely disappear in a long dry period, although they quickly multiply shortly after the return of rain. Animals, however, which are able, usually burrow during a period of drought to a greater depth where moisture lingers for a longer time.

Estimations of the available water content of the soil have been carried out throughout the investigation and the results plotted. This water content will naturally vary in different parts of the field according to the soil components and every slight change in slope.

To obtain an appropriate sample, the soil was taken near the line along which the faunal investigation was carried out, and in order that the samples might be representative, a thin slice of earth of about six inches depth was taken from each of several spots and these were placed together in an air-tight jar, thoroughly mixed and the available water content calculated.

The water content percentages obtained are shown graphically in Fig. 2 together with curves for the soil population. The figure shows that the percentages recorded vary within considerable limits in a comparatively short space of time. Neither water content nor faunal samples were taken at a particular period during December 1920, when a fairly heavy fall of snow occurred which froze into a hard mass; the top two inches or so of the soil were also frozen, the temperature at one time being 2° C. below zero. The next sample taken after this period showed a marked fall in the totals for the fauna.

The maximum water content also varied with every slight difference in the components of the soil and it was found to increase in direct proportion to the amount of humus present. The same method was adopted as above to obtain a representative sample, though even so, the percentages obtained differed within considerable limits. The average, however, worked out to 81·5 per cent.

The pore space of the soil (average 48·9 per cent.) varied likewise, as it is affected by the presence of organic and colloidal matter (16) besides being initially dependent on the packing of soil particles of different sizes. On the other hand, the results of estimations for hygroscopic moisture showed no marked variability, giving an average percentage of 3·45.

The humus content of the soil varied with depth:

1–3	inches depth	14·2	per cent.
3 6	,,	14·5	,,
6–9	,,	10·0	,,

Throughout the greater part of the investigation, weekly temperature readings were taken in the atmosphere (in the shade) and at depths of 4½ and 9 inches. The data recorded show the following points:

i. That there is a decided difference between temperatures at different depths.

ii. That the temperature of the soil varies in accordance with that of the atmosphere. The two periods at which the atmosphere and soil curves separate notably from one another are marked by very sunny days.

iii. That the soil temperature varies less than that of the atmosphere, the latter being higher than that of the soil on warm days and lower on very cold days.

iv. That, generally speaking, the soil at 9 inches depth is warmer than that at 4½ inches. Consequently the fauna would find a certain advantage in burrowing deeper into the soil during excessively cold periods.

A mechanical analysis of representative samples of the soil gave the following table of results:

	Maximum and minimum size of particles mm.	Percentage
Fine gravel	3·1	22·36
Coarse sand	1–0·2	15·96
Fine sand	0·2 –0·4	13·34
Silt	0·04 –0·01	12·80
Fine silt	0·01 –0·002	18·60
"Klay"	0·001–below	11·88
Loss on treatment with HCl		3·25.
Hygroscopic moisture		3·45.

Full chemical analyses were not made, but it was apparent that the amount of carbonates in the soil was negligible; that iron was present to a marked degree, and that lead though present in the district was not detected in this soil by the ordinary tests.

(ii) THE SOIL FAUNA. The term Soil Fauna is used to include all members of the animal kingdom, other than microscopic forms, occurring actually within the soil. Any forms found on the surface are included providing they may also occur beneath the soil, e.g. *Collembola*, *Coleoptera*, and so on.

Representatives of the following divisions were present: *Nematoda*, *Oligochaeta*, *Crustacea*, *Arachnida*, *Myriapoda*, *Insecta*, *Mollusca*.

Of the above, the sub-groups *Collembola* and *Acarina* were the best represented in point of numbers of individuals, and the *Coleoptera* in variety of species.

When following the quantitative changes in the fauna of an area under varying conditions throughout the year, it is important to attempt some estimate of any statistical error that may be involved by the method of collecting the data. One requires some knowledge as to the horizontal distribution of the fauna, and some indication as to whether the standard sample taken is of sufficient size. An effort has been made to test the value for quantitative purposes, of the data presented, in the following way.

i. On each of several occasions 20 small samples of soil, each a cube of two inches, were taken at random from all over the field, the only condition being an apparently similar covering of vegetation in each case. The number of *Achorutidae* present were counted for each sample, as an index of the total census.

The results showed a great range in numbers within each series of 20 countings, being for example in one series from 5 to 22, and one may conclude that a cube of two inches is an unsatisfactorily small sample. However, the totals of the several series (192, 193, 194, 196, 199, 199, 201, 204, 204) were comparable, suggesting that a cube of 9 inches is a sufficiently large sample.

ii. Similar two-inch cubes of soil were taken according to a methodical plan, namely along parallel lines, running N.E. to S.W., at a distance of 20 feet from each other, the samples being spread along the lines 12 feet apart.

The totals for these series agreed very well with the totals obtained above (194, 194, 194, 195, 195, 197, 198, 200, 200), the individual countings for each small sample as before showing a wide range.

A series of similarly placed samples from lines running N.W. to S.E. gave totals 179, 190, 190, 190, 194, 196, 196, 199, 199. Only in the first total above, of all the series taken, was there an absence of high counts.

iii. Lastly, 20 two-inch cubes were removed from the surface of three normal nine-inch samples. Once more the high variability of the individual counts was noted, and totals comparable with those obtained previously (201, 201, 204).

The totals of all these series of samples range as follows: 179, 190, 190, 190, 192, 193, 194, 194, 194, 194, 194, 195, 195, 196, 196, 196, 197, 198, 199, 199, 199, 199, 200, 200, 201, 201, 201, 204, 204, 204, and if one omits the first of these the similarity of the others is evident.

It was concluded that the examination of a nine-inch cube of soil might be expected to give a reasonable picture of the Achorutid population of the soil as a whole and that such a sample was a reasonable one on which to work in attempting to trace quantitatively the seasonal changes of the fauna. Samples of this volume were examined from June 1920 to October 1921, inclusive, but for the remaining months it was thought to be sufficiently instructive to deal with the surface three inches of soil only.

The census of the fauna obtained throughout the investigation is set out in the following tables and some of the data from these tables are summarised and represented graphically by the curves in Figs. 3 and 4. The columns in Fig. 4 show the relative frequency of occurrence of the individual groups.

SPECIFIC DISTRIBUTION OF THE SOIL FAUNA.

The Seasonal Census.

The formula used in the following list is based on that used by Morris (20). For example:

June 1920-1.

Lumbricus terrestris $\dfrac{\text{A } 72 : 10\ (8)}{6, \text{ etc. to } 5} : \dfrac{\text{B } 14}{7, 8, 10, 11, 1, 5}$.

A = surface three inches of soil: 72 = total number of individuals found in the samples examined between June 1920 and June 1921 (average—two samples per month): 10 = the maximum number of individuals which occurred in any one sample: (8) = the month in which this maximum occurred, that is, August. Below the line "6, etc. to 5" indicates the months in which *L. terrestris* was found. That is, in every month in which samples were examined. Similarly, *B* represents the remaining layers of soil in the samples, that is, from three to nine inches depth: 14 = the total number of individuals found therein: "7, 8, 10..." the months in which they occurred, that is July, August, October.... As the total numbers are so much smaller in B, the maximum total in any one sample has not been included.

The letter (or letters) in brackets placed after the name of a species, e.g. *Dilophus Febrilis* L. (L) indicates the stage of development, *i.e.* (L) = Larva or (LPA) = a species found in larval, pupal, and adult stages, or (C) = cocoon.

DISTRIBUTION OF THE SOIL FAUNA OF PASTURE LAND.

[Average—2 samples per month]

	June 1920-1 (Sept. and Dec. not included)		June 1921-2 Surface 3 ins. only*
NEMATODA			
Nematoda spp.	A 321 : 139 (10) 6, 7, 8, 10, 11, 4, 5	: B 32 6, 7, 10, 11	346 : 56 (12) 8, 9, 10, 11, 12, 1, 2, 3, 4, 5
Mermithidae including Mermis brevis Hagmeier	A 15 : 4 (11) 7, 10, 11, 1, 3, 5	: B 46 6, 7, 8, 10, 11, 1, 4	6 : 3 (4) 3, 4, 5
OLIGOCHAETA			
Small white forms including Fredericia unibaelsenia, F. diabeta, F. bisetosa Ler., F. perrieri Enchytraeids including E. albidus, etc.	A 593 : 86 (10) 6, etc. to 5	: B 51 6, 7, 8, 10, 11, 1, 2, 4, 5	43 : 8 (1) 6, 9, 10, 11, 12, 1, 2, 3, 4
Lumbricus terrestris	A 72 : 10 (8) 6, etc. to 5	: B 14 7, 8, 10, 11, 1, 5	80 : 14 (9) 6, 8, 9, 10, 11, 12, 1, 2, 3, 4, 5
L. costaneus	A 3 : 2 (2) 1, 2	: —	1 1
Helodrilus oculatus	A 12 : 4 (8) 8, 10, 11, 1	: B 1 6	2 2, 5
Allolobophora chlorotica	A 8 : 2 (10) 6, 7, 8, 10, 11, 1	: —	15 : 4 (1) 8, 9, 1, 3, 4

* For five months of this year samples were examined to a depth of 9 inches (see Fig. 2), but for convenience the population of the first 3 inches only is given for June 1921-2. So that in the text the total numbers refer to the figure rather than the tables.

[Average—2 samples per month]

	June 1920-1 (Sept. and Dec. not included)			June 1921-2 Surface 3 ins. only
ISOPODA				
Porcellio scaber	A 1 3	:	—	1 7
ARACHNIDA				
(*a*) *Acarina*				
(1) Gamasidae				
Pergamasus crassipes Linn.	A 127 : 22 (11) 6, etc. to 5	:	B 15 7, 10	156 : 40 (12) 6, 8, 9, 10, 11. 12, 1, 2, 3, 4, 5
var. longicornis	A 3 8	:	—	—
P. septentrionalis Oud.	A 29 : 7 (11) 11, 1, 3, 4, 5	:	—	11 : 2 (11, 4, 5) 8, 11, 1, 2, 4, 5
P. rumiger Berl.	A 1 11	:	—	—
P. meridionalis Berl.	A 1 11	:	—	—
P. runcatellus Berl.	A 1 2	:	—	—
Oligogamasus pollicipatus	A 21 : 7 (1) 11, 1, 2, 3, 5	:	B 1 5	10 : 3 (10) 7, 10, 11, 1, 2, 3, 5
Gamasus fimetorium Berl.	A 7 : 2 (8, 10) 6, 7, 8, 10, 1	:	B 1 10	20 : 10 (10) 6, 10, 11, 1
G. magnus Kr.	A 13 : 4 (11) 10, 11, 4, 5	:	—	10 : 3 (3, 5) 3, 5
Pachylaelops sp.	A 34 : 10 (11) 10, 11, 1, 2, 3, 4, 5	:	B 4 10, 1, 5	52 : 9 (1) 7, 8, 9, 11, 12, 1, 2, 3, 4, 5
Zercon perforatulus Berl.	—	:	—	3 : 2 (1) 1, 3
Dinychus tetraphyllus Kr.	A 1 4	:	—	4 : 3 (2) 1, 2
Spp. Nymphs	A 61 : 10 (10) 7, 8, 11, 1, 4, 5	:	B 5 7, 10	298 : 34 (1) 6, 8, 9, 10, 11, 12, 1, 2, 3, 4, 5
(2) Tyroglyphidae				
Rhizopus echinopus Rob.	A 12 : 4 (8) 8, 10, 11, 4, 5	:	B 1 10	11 : 3 (1) 11, 1, 3, 5
(3) Thrombidiidae				
Bdella lignicola Can.	A 3 : 2 (11) 11, 1	:	—	—
B. lacustris Hull.	A 3 4, 5	:	—	6 : 3 (6) 6, 9, 5
Enemothrombium subrasum Berl.	A 5 : 2 (11) 7, 10, 11, 1	:	—	1 11
(4) Oribatidae				
Archipteria redux Hull.	A 1067 : 150 (6) 6, etc. to 5	:	B 51 7, 8, 10, 4	1893 : 184 (5) 6, etc. to 5
Notaspis lacustris Michael	A 4 6	:	—	5 : 3 (9) 7, 9, 1

	[Average—2 samples per month] June 1920-1 (Sept. and Dec. not included)			June 1921-2 Surface 3 ins. only
Arachnida (*contd.*)				
(*b*) *Aranĕae*				
(1) Ciniflonidae				
Ciniflo atrox	A 4 : 2 (5) 1, 5	:	—	5 : 3 (5) 5
(2) Linyphidae				
Walckenaera punctata	A 5 : 3 (1) 10, 1, 5	:	—	3 : 2 (6) 6
W. pratensis	A 1 10	:	—	3 : 2 (6) 6
(3) Epëiridae				
Epëira diadema	—	:	—	1 7
Myriapoda				
(*a*) *Symphyla*				
Including spp.: Scolopendrellopsis subnuda Hans. Symphylella delicatula Bagn. S. vulgaris Hans.	A 33 : 12 (5) 6, 8, 10, 11, 1, 2, 3, 4, 5	:	B 106 6, etc. to 5	55 : 9 (5) 6, 7, 8, 11, 2, 4, 5
(*b*) *Diplopoda*				
Julus varius	A 1 1	:	—	—
J. sp.	A 2 5	:	—	—
Sp.	—	:	—	1 11
(*c*) *Chilopoda*				
Geophilus longicornis Leach	A 8 : 2 (2) 7, 11, 1, 2, 5	:	B 8 7, 8, 10, 11, 1	2 1, 5
Lithobius erythrocephalus	—	:	B 1 6	—
L. forficatus	—	:	B 1 2	—
Brachygeophilus truncorum	—	:	B 2 1	—
Insecta				
(*a*) *Collembola*				
(1) Achorutidae				
Onychiurus ambulans Linn.	A 212 : 45 (2) 6, etc. to 5	:	B 148 6, etc. to 5	1288 : 155 (4) 6, etc. to 5
O. armatus (Tulb.)	A 343 : 123 (11) 6, etc. to 5	:	B 209 6, etc. to 5	318 : 49 (11) 6, etc. to 5
O. fimetarius Linn.	A 131 : 58 (11) 7, 10, 11, 1, 2, 3, 4, 5	:	B 62 6, 8, 10, 11, 1, 3	5 : 4 (1) 6, 1
O. Burmeisteri (Lubb.)	A 11 : 7 (2) 2, 4	:	B 5 2	1 7
Achorutes armatus (Nic.)	A 10 : 3 (2, 5) 8, 10, 2, 5	:	B 1 2	905 : 488 (3) 6, 7, 9, 10, 11, 1, 2, 3, 4, 5

	[Average—2 samples per month] June 1920-1 (Sept. and Dec. not included)			June 1921-2 Surface 3 ins. only
INSECTA (*contd.*)				
A. purpurescens Lubb.	—	:	—	1 11
Tullbergia quadrispina (Börn.)	—	:	—	47 : 23 (11) 11, 1, 2, 3, 4, 5
(2) Entomobryidae				
Entomobrya muscorum (Nic.)	A 5 : 2 (11, 4) 11, 4	:	—	23 : 12 (8) 8, 1, 4
E. multifasciata (Tulb.)	A 6 : 4 (4) 11, 4	:	B 1 2	1 3
E. Nicoleti (Lubb.)	A 33 : 10 (11) 11, 1, 3, 4, 5	:	B 2 1	20 : 8 (5) 8, 9, 11, 5
Isotoma viridis Bourl.	A 260 : 125 (2) 6, 7, 10, 11, 1, 2, 3, 4, 5	:	B 9 11, 1, 2, 3, 4, 5	854 : 139 (1) 6, etc. to 5
I. plumbea Lubb.	A 2 1	:	—	1 5
I. anglicana Lubb.	A 10 : 9 (2) 1, 2	:	—	2 2, 3
I. grisescens Schäff.	A 4 7	:	B 1 7	3 11
I. viatica Lubb.	A 1 1	:	—	1 6
Isotomurus palustris (Müll.)	A 80 : 18 (11, 1) 7, 8, 10, 11, 1, 2, 3	:	—	35 : 12 (5) 1, 2, 3, 4, 5
var. prasina Reut.	A 16 2	:	—	—
Beckia argentea Lubb.	A 13 : 5 (10) 8, 10, 11, 1, 4	:	B 2 1, 2	—
B. albinos Nic.	A 2 10, 1	:	—	5 8
Folsomia fimetaria Tulb.	A 51 : 32 (2) 6, 7, 8, 1, 2, 4, 5	:	B 14 1, 2, 3	716 : 145 (5) 6, etc. to 5
(3) Sminthuridae				
Sminthurus viridis Lubb.	A 10 : 6 (5) 2, 5	:	B 5 2, 5	8 : 3 (7) 6, 7, 9, 1, 5
S. aureus var. ochropus Reut.	A 2 11, 5	:	—	—
S. luteus Lubb.	A 3 : 2 (11) 11, 1	:	B 1 5	20 : 12 (6) 6, 3, 4, 5
S. niger (Lubb.)	A 9 : 2 (4, 5) 11, 4, 5	:	—	4 : 2 (11) 11, 4
Bourletiella hortensis (Fitch.)	A 1 6	:	B 1 8	2 6, 7
*"Larvae"	A 160 : 29 (10) 8, 10, 11, 1, 3, 4, 5	:	B 77 6, 7, 10, 1, 2, 3, 4, 5	1725 : 181 (5) 6, 9, 10, 11, 12, 1, 2, 3, 4, 5
(*b*) *Rhynchota*				
(1) Heteroptera				
Calocoris sp. (N)	A 1 6	:	—	—

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* The arbitrary term "Collembolan larvae" is used to include such forms of the group as are too immature for specific identification.

	[Average—2 samples per month] June 1920–1 (Sept. and Dec. not included)			June 1921–2 Surface 3 ins. only
Insecta (*contd.*)				
(2) Homoptera				
Myzus sp. (AN)	—	:	—	14 : 10 (10) 7, 9, 10, 2, 5
Sp. (N)	A 2 2	:	—	6 : 2 (7, 8) 7 8, 11
(3) Psyllidae				
Psylla sp. (A)	A 1 2	:	—	—
(*c*) *Thysanoptera*				
Physothrips consociata (A)	A 2 6	:	—	1 5
(*d*) *Lepidoptera*				
Spp. (3) (L)	—	:	—	3 : 2 (11) 11, 5
(*e*) *Coleoptera*				
(1) Carabidae				
Amara communis Panz. (A)	A 4 7, 11, 1, 2	:	—	1 9
Bradycellus verbasci Duft. (A)	A 1 11	:	—	—
Nebria brevicollis F. (L)	A 7 : 2 (7, 10) 7, 10, 11, 1	:	B 1 1	2 8
Spp. (3) (L)	A 1 2	:	—	7 : 6 (6) 6, 11
(2) Hydrophilidae				
Megasternum boletophagum Marsh (A)	A 1 5	:	B 1 4	3 : 2 (9) 6, 9
(3) Staphylinidae				
Xantholinus linearis (A)	A 2 10, 5	:	B 1 10	19 : 5 (10, 5) 7, 9, 10, 11, 1, 3, 4, 5
X. sp. (L)	A 2 10	:	—	4 : 2 (6) 6, 5
Ocypus olens Müll. (A)	—	:	—	1 11
O. cupreus Rossi. (A)	A 3 : 2 (6) 6, 7	:	—	2 5
Philonthus or Quedius sp. (L)	A 2 6, 1	.	—	17 : 6 (5) 6, 7, 11, 1, 3, 4, 5
Homalota fungi Gr. (A)	—	:	—	1 9
Spp. (2) (L)	A 1 5	:	—	5 : 3 (11) 11, 2
(4) Pselaphidae				
Euplectus kunzei Aubé (A)	—	:	—	1 7
(5) Trichopterygidae				
Trichopteryx sp. (A)	A 3 6, 1, 3	:	B 1 8	1 8
Ptenidium evanescens Marsh (A)	A 1 6	:	—	—

	[Average—2 samples per month] June 1920–1 (Sept. and Dec. not included)			June 1921–2 Surface 3 ins. only
INSECTA (*contd.*)				
(6) Byrrhidae				
Byrrhus pilula L. (L)	A 8 : 3 (11, 5) 11, 2, 5	:	—	1 4
(7) Lathridiidae				
Corticaria fulva Com. (A)	A 1 5	:	—	—
(8) Lucanidae				
Sp. (L)	A 1 2	:	—	—
(9) Scarabaeidae				
Aphodius fossor L. (A)	—	:	—	1 1
Aphodius punctatosulcatus Sturm (A)	—	:	—	1 11
(10) Elateridae				
Agriotes obscurus L. (L)	A 45 : 6 (4) 6, 7, 10, 11, 1, 2, 3, 4, 5	:	B 1 5	26 : 6 (12) 6, 8, 11, 12, 1, 2, 3, 5
A. lineatus L. (L)	A 6 : 2 (7) 6, 7, 10, 11	:	—	—
Athous haemorrhoidalis F. (L)	A 3 : 2 (7) 7, 8	:	—	—
(11) Chrysomelidae				
Longitarsus luridus Scop. (A)	A 1 3	:	—	—
L. gracilis Kuts. (A)	A 1 5	:	—	—
(12) Crioceridae				
Lema melamopa (A)	A 2 6	:	—	—
(13) Curculionidae				
Sitones flavescens Marsh (A)	—	:	—	1 6
S. hispidulus F. (P)	A 1 2	:	—	1 5
Hypera punctata F. (A)	—	:	—	2 8, 5
Rhinonchus pericarpius L. (A)	—	:	B 1 5	1 11
Lissoma ovatulum Clairv. (A)	—	:	—	1 11
Sp. (L)	A 13 : 3 (4, 5) 11, 1, 2, 3, 4, 5	:	B 1 5	57 : 7 (11) 6, 7, 8, 9, 10, 11, 1, 3, 4, 5
(14) Helopidae				
Helops strictus (L)	A 1 2	:	—	—
(*f*) *Diptera*				
(1) Cecidomyiidae				
Cecidomyia buxi (P)	A 1 6	:	—	—
C. destructor Say. (P)	A 2 5	:	—	—

	[Average—2 samples per month] June 1920–1 (Sept. and Dec. not included)			June 1921–2 Surface 3 ins. only
INSECTA (*contd.*)				
C. sp. (L)	A 4 : 2 (1) 1, 5	:	—	2 6
(2) Mycetophilidae				
Sciara sp. (L)	A 17 : 6 (2) 11, 1, 2, 4	:	—	—
(3) Bibionidae				
Dilophus febrilis L. (L)	A 132 : 96 (1) 11, 1, 3	:	—	71 1
Bibio Johannis L. (L)	A 1 2	:	—	—
(4) Tipulidae				
Tipula paludosa Mg. (L)	A 10 : 2 (1, 3) 10, 1, 2, 3, 5	:	—	15 : 5 (10) 8, 9, 10, 11, 2, 5
T. sp. (L)	—	:	—	15 : 5 (4) 1, 2, 3, 4, 5
(5) Leptidae				
Leptis scolopacea L. (L)	A 39 : 9 (11) 6, 7, 8, 10, 11, 1, 5	:	B 6 6, 7, 10, 11	6 : 2 (9) 9, 10, 1, 2, 4
L. tringaria L. (L)	A 4 11, 1, 2, 5	:	B 2 11, 1	1 6
(6) Empidae				
Rhamhomyia sulcata Fln. (P)	A 2 4, 5	:	B 2 4	—
(7) Cordyluridae				
Scatophaga stercomaria L. (P)	—	:	—	1 1
(8) Anthomyiidae				
Hyetodesia incana W. (L)	A 20 : 5 (1) 8, 10, 11, 1, 5	:	B 1 4	2 3, 5
Coenosia sp. (P)	A 1 5	:	—	—
Hydrotoea irritans Fln. (P)	A 6 : 5 (5) 4, 5	:	—	17 : 3 (5) 6, 11, 1, 2, 4, 5
(9) Chironomidae				
Tanypus maculatus (L)	A 3 : 2 (1) 11, 1	:	—	—
(10) Phoridae				
Phora sp. (P)	A 4 : 2 (2) 6, 2, 5	:	—	15 : 4 (4) 7, 8, 11, 12, 1, 3, 4
(11) Spp. (3) (LP)	A 6 : 3 (2) 11, 2, 3, 5	:	B 1 6	6 : 2 (9, 5) 7, 9, 1, 5
(*g*) *Hymenoptera*				
Aphidius danci (C)	A 3 : 2 (6) 6, 4	:	—	—
Myrmica scabrinodis (APL)	—	:	—	273 7
Pteromalinae-Chalcidae				
Sp. (A)	—	:	—	2 6, 7

	[Average—2 samples per month] June 1920–1 (Sept. and Dec. not included)			June 1921–2 Surface 3 ins. only
INSECTA (*contd.*)				
(*h*) *Mollusca*				
Limax agrestis	A 7 6, 10, 11, 1, 2, 5	:	—	—
L. sp.	A 3 $\overline{8}$, $\overline{11}$, 5	:	—	2 1, 2
Arion ater	A 1 10	:	—	—
Planorbis sp.	A 1 $\overline{10}$	:	—	3 $\overline{8}$

Fig. 2 shows the quantitative seasonal changes in the total fauna for each of the three specified depths of soil. It is evident that the population of the first three inches is, with rare exception, markedly greater than that of lower depths, and that for the most part, the general rise and fall of the curves is fixed by this "upper" population. The upper curve shows throughout an irregular rise and fall, but in addition it has a well-marked trend, being high in winter and low in summer.

The difference in the levels of the curve during summers 1920 and 1921 is very marked and has every appearance of being related to the unusual precipitations and unusual dryness, respectively, of these two years. The dry conditions of 1921 certainly caused many organisms to die off, their shrivelled bodies being found in considerable quantities.

Points on the curves marked *, †, ⊙, ⁎, × indicate the occurrence of large colonies of organisms which are not included in the totals from which the curve was drawn, as they were not typical of a sample, but only occurred scattered here and there over the field. These colonies did not appear to have any influence upon the rest of the fauna or flora of their immediate neighbourhood.

The census curves for the depths three to six inches and six to nine inches follow each other very closely; their respective populations do not materially differ and the numbers are small. In no case does the population of either of these lower depths exceed that of the surface three inches. Just as the curve for the total fauna is determined by the fauna of the upper three inches, so that for the upper three inches is itself determined by the prevalence of *Collembola* and *Acarina* (Fig. 3).

The soil was examined, occasionally, below the specified depth of the routine samples. Under normal or very moist conditions organisms were of very rare occurrence below nine inches; an occasional *Achorutid*

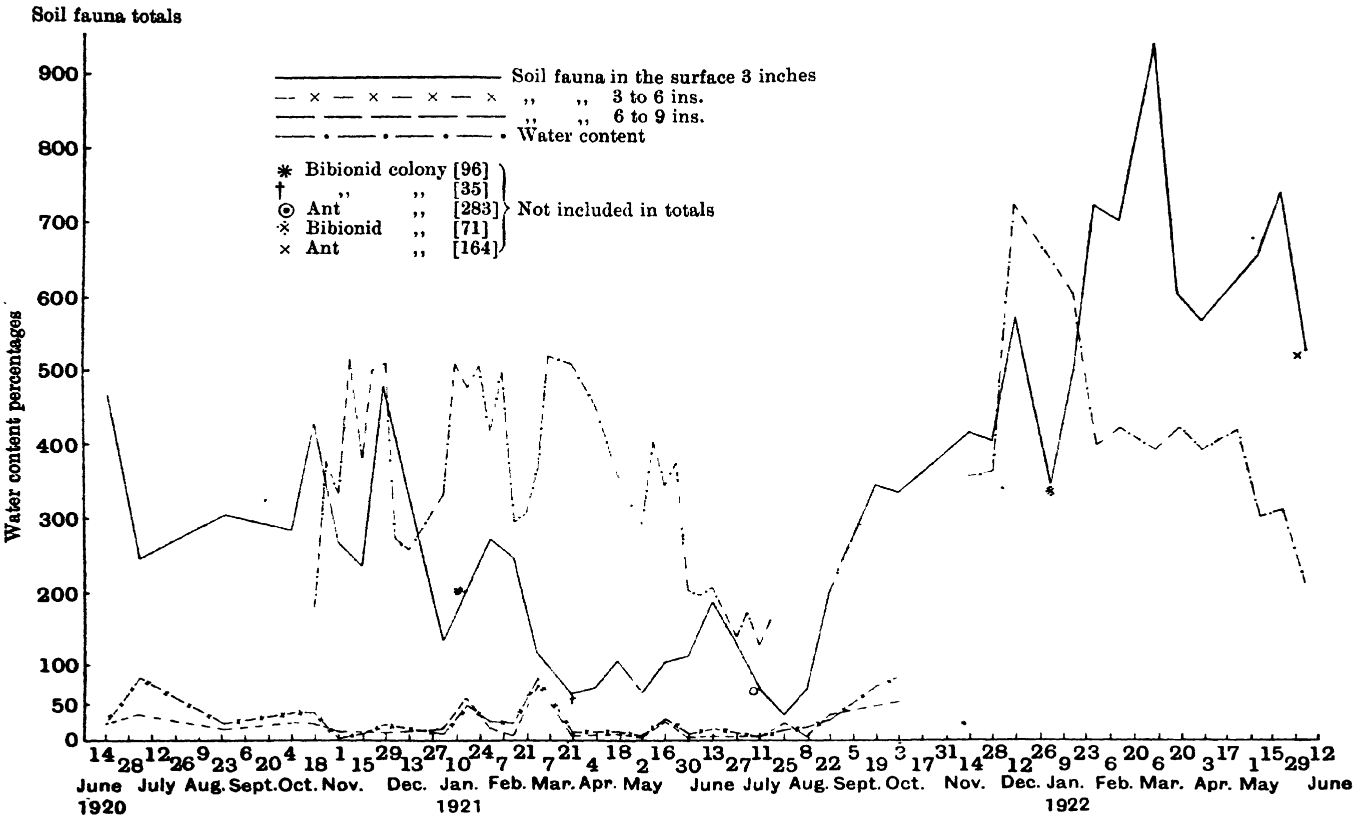

Fig. 2. Distribution of the soil fauna—Pasture land.

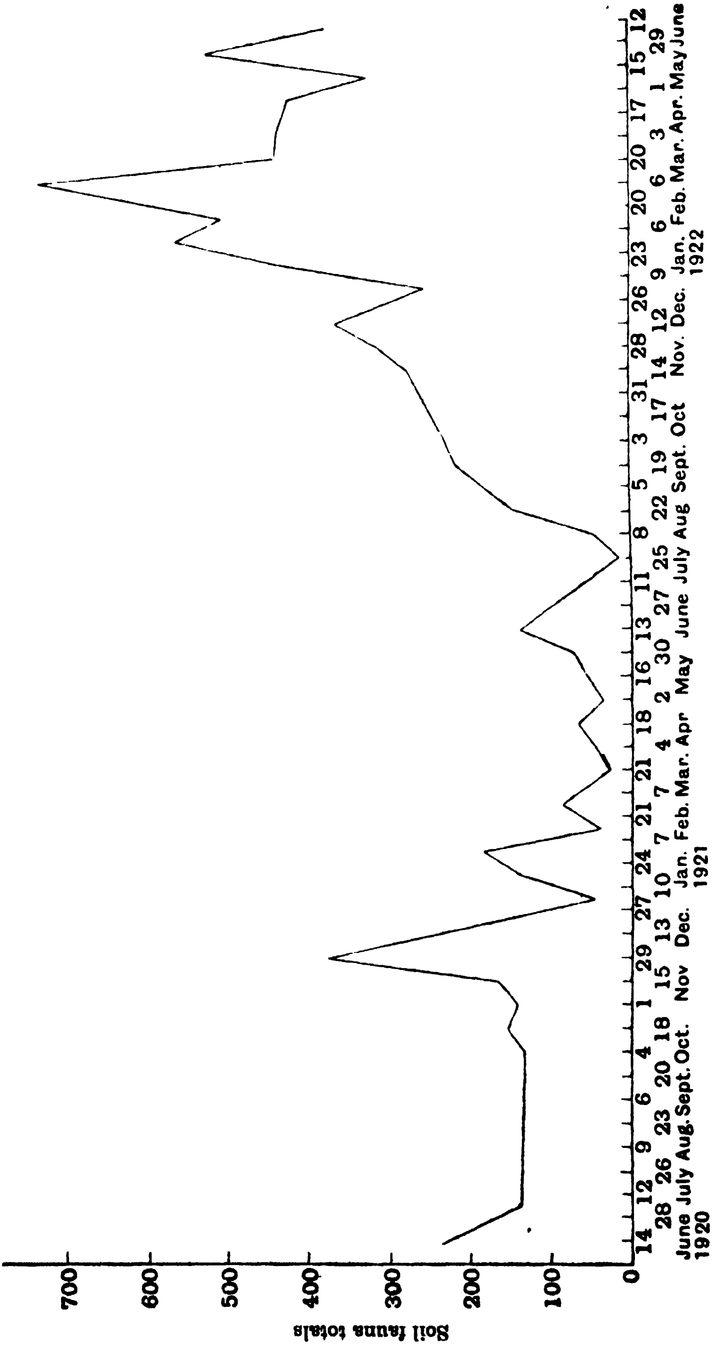

Fig. 3. Curve of the totals of Achorutidae, Collembolan larvae and Acarina [in the surface three inches of soil].

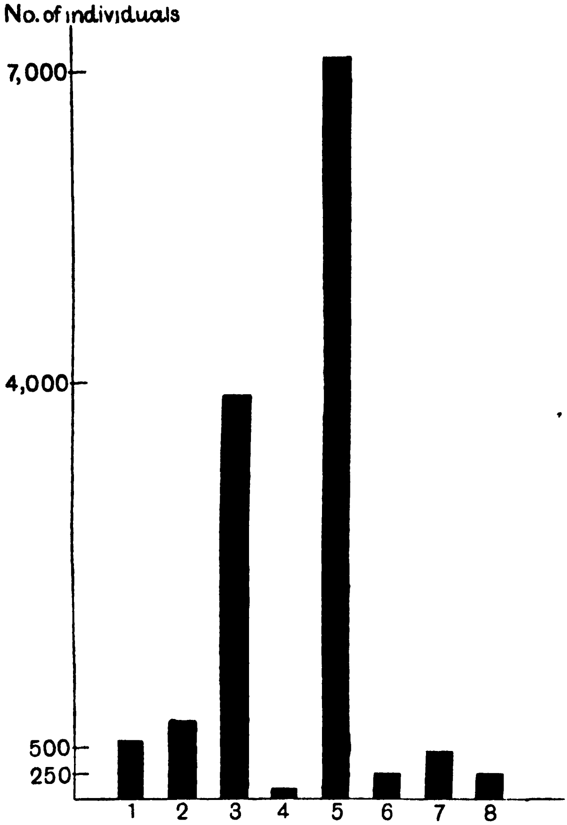

Fig. 4. *Pasture.* Relative frequency of occurrence of individual groups in the surface three inches of soil.

Scale—1 cm. = 500 individuals.

1, Nematoda; 2, Oligochaeta; 3, Acarina; 4, Myriapoda; 5, Collembola; 6, Coleoptera; 7, Diptera; 8, Hymenoptera.

Totals of other groups are too small to be included in the diagram.

was found. As the soil became dry, *Oligochaetes* began to work their way downwards, and when drought conditions held, a few *Symphyla* and *Achorutidae* and an occasional insect larva occurred at a depth of from nine to twelve inches, but not lower.

The curve for the water content of the soil has been included in Fig. 2; it is based on the percentages occurring either weekly or on the days on which the samples were taken. There is some suggestion that the water content of the soil may offer an explanation of the variation of the faunal census, though this is by no means proved. Certainly the effect of moisture on *Collembola* and small white *Oligochaetes*, at least, is very marked.

Of the *Insecta* the group *Collembola*(17) was most abundantly represented in the soil fauna as regards the number of individuals and showed a fairly wide variety in species. They were ubiquitous, occurring vertically and horizontally throughout the samples. Whilst far more numerous in the upper three inches, it is rather surprising that a considerable number of these delicate organisms should occur at comparatively low depths. In these lower parts of the soil the majority of the forms found were representatives of the *Achorutidae*, in which family the saltatory apparatus is poorly developed or missing. Less frequently "springing" forms were also present, though in this position the "spring" could not function in the usual manner.

The number of individuals per sample varied very considerably, for example, from 5 to 543 in the upper three inches. Very low numbers were not common, and when these occurred, they followed a spell of exceedingly cold or very dry weather.

Experiments were carried out with regard to the length of Collembolan life under artificial drought conditions. A number of healthy adult *Achorutidae* were placed in a shallow dish, the water content of the soil being approximately 36 per cent. The dish was left exposed to the air of the laboratory, the temperature of which was 40° F. The soil dried in the night, leaving only a certain amount of hygroscopic moisture. On examination of the organisms on the following day less than 50 per cent. were found alive and all had succumbed by the third day.

The extremely small number of individuals, five only, found in the sample January 3rd, 1921, might be explained as being related to the preceding severe cold and low water content during December 1920, for at the end of November, the numbers reached 252. There was again an increase to 149 on January 31st, 1921, consisting of larvae and adults. This seems to imply that a new generation had appeared, hatched from eggs which had resisted the inclement conditions. According to Lubbock(17) "the eggs are laid and the young hatched apparently within a period of from six to ten days."

The food of these Collembolans seemed to be humus, though a few specimens were noted which appeared to be nibbling the turf roots. As stated by Theobald(27), they can do sufficient damage to open up plant tissues to fungal or bacterial attack; those species which feed on the attacking fungi or bacteria are probably carriers of the disease. Species known to be injurious and which occurred in this area are as follows: *Onychiurus ambulans, O. armatus, O. fimetarius, O. Burmeisteri, Achorutes* *armatus, A. purpurescens, Isotomurus palustris, Entomobrya multifasciata, Sminthurus luteus, Bourletiella hortensis.* No damage to the vegetation, however, has been recorded in the present investigation, and in no case was there evidence of attack by Collembolans on animal forms.

The most common form of parasitic attack upon Collembolans was that by small Nematodes, which pierced the abdomen, usually between the second and third segments. In some cases the Nematodes were wholly internal, coiled round the organs, but when large they appeared to penetrate the organs, leaving half the body projecting freely from the host. Throughout the time they were under observation the position of the parasite did not alter and the hosts were capable of slow dragging movements. In the cases noted the attack was confined to members of the *Achorutidae.*

Bacteria were present in the majority of specimens examined, apparently not harmful types.

Ecdyses of the *Collembola* were evidenced by the presence of perfect specimens of whitish cast skins in the soil at varying depths. With two exceptions, they occurred in each month during the course of the investigation, being found mainly in the upper three inches of soil. The maximum numbers of cast skins occurred in June 1920, September 1921, January, April and May 1922.

The three families *Achorutidae, Entomobryidae* and *Sminthuridae* were represented by 7, 12 and 5 species respectively. Four of the 24 species, however (*Achorutes armatus, Onychiurus ambulans, O. armatus* and *Isotoma viridis*) contributed approximately 58·5 per cent. of the total number of adults (7176) found during the investigation in the first three inches of soil. In the lower layer of soil, the fall in numbers was very marked; this is probably due to the concentration of the food supply in the first two or three inches of soil (humus and roots) and possibly the better aeration of the soil near the surface. The species in the lower layer were similar to those in the upper though with less variety in the *Entomobryidae.*

Owing to the relative inactivity of the *Achorutidae* and the considerable numbers in which they occur, these organisms form excellent material for quantitative study. The family contributed the greater part of the Collembolan population at all depths in the soil. In no case did they appear to form definite colonies, but were fairly evenly distributed.

Entomobryids contributed but little to the composition of the fauna at depths below three inches; of the 12 species recorded, *Isotoma viridis* was by far the most abundant, varying in size and colour to a great extent. Since the springing organ in this family is strongly developed, some individuals may have escaped during the collection of the samples, but it is thought that on the whole, the figures convey a fair idea of quantitative occurrence.

Throughout the investigation it was noted that *Isotoma viridis* and *Isotomurus palustris*, or *Isotoma viridis* and *Folsomia fimetaria*, were usually found together, and *Onychiurus ambulans* and *O. armatus* almost invariably so.

The *Sminthuridae* were at all times of rare occurrence and were found typically among the bases of grass tufts. They appeared to feed on living vegetation though were not present in sufficiently large numbers to cause any perceptible damage. The largest form, *Sminthurus viridis*, was the most common species.

Larvae contributed approximately 23 per cent. of the total Collembolan individuals.

The group *Rhynchota* was poorly represented and the individuals which occurred belonged mainly to the *Homoptera*. *Aphid* nymphs were the most frequent, a few individuals being sufficiently advanced to be referred to the genus *Myzus*. One individual appeared to represent a new species of this genus. The only *Heteropteran* present was a nymph in a very early stage of development; probably *Calcoris* sp. With one exception, the above individuals all occurred immediately beneath the surface.

Representatives of the *Thysanoptera* and *Lepidoptera* occurred very rarely, seven specimens only being found in the total number of samples.

Coleopterans (11, 22) were fairly well represented in point of species (35) though the total number of individuals recorded (280) was not great. The only species found with any frequency were *Agriotes obscurus* and *Weevil* larvae. Together these contributed a very considerable proportion of the total *Coleopteran* population. Recognised pests were present, notably *A. obscurus* and *Weevil* larvae, but no particular damage was noted for this area.

Representatives of 14 families occurred, adults and larvae, but the latter were always more frequent. Owing to the difficulty of rearing *Coleopterous* larvae to the adult stage for identification, many cannot be referred to more than their genus or even family. The larvae occurred almost exclusively within the upper three inches of soil; this is probably explained by the fact that the majority are root feeders and the level of the root area ends, for the most part, 2½ to 3 inches down. Carnivorous forms would also tend to concentrate here.

The larva of *Nebria brevicollis* was the most commonly occurring form, usually found in connection with the Elaterid *Agriotes obscurus*. One individual was captured holding two small specimens of *Onychiurus ambulans* between its mandibles, tenaciously clinging to them even when disturbed; these may or may not form part of their regular diet.

Amara communis may be merely a temporary invader of the area, as the adults show a marked habit of flying from place to place and only penetrate the soil very superficially; also, no larvae were found.

A rare form recorded was *Euplectus kunzei*.

More species were recorded for the *Staphylinidae* than for any other family (8), *Xantholinus linearis* adults and *Quedius* (or *Philonthus*) sp. larvae being the most common, both of which appeared in the lower layers of soil as well as in the upper.

The Criocerid *Lema melamopa* is interesting in that while it often occurs on the leaves of rye grasses and other Gramineae, in this investigation specimens were found free in the soil; possibly the eggs may be laid underground.

Larval forms of *Curculionidae* were fairly abundant and usually found round the roots of *Dactylis glomeratas*.

Diptera (28,29) were well represented (20 species) though much less so than the previous group. The total number of individuals was, however, increased by the occasional occurrence in the samples of large colonies of larvae of the Bibionid *Dilophus febrilis*; these occurred in three samples and gave totals of 96, 35 and 71 individuals respectively. When reared the adults usually emerged two months earlier in the laboratory than they would have done under normal conditions.

Tipulid larvae appeared to feed on all roots irrespective of species and larvae of *Leptis scolopacea* exclusively on humus or the rotting bases of grass sheaths or leaves, though its family the *Leptidae* is notoriously predaceous.

Tanypus maculatus was remarkable in its occurrence in normal pasture land, it being typically found in water; here it occurred over 200 feet from the stream traversing the neighbouring pasture field. All the characteristic features were fully developed. During summer months, the adults of this family were often found entangled among the grasses of the samples as were also those of *Phoridae* and *Cecidomyiidae*; only the immature forms of the families are, however, included in the census.

The group *Hymenoptera* was not well represented in the samples, the most notable instances being the occurrence of large colonies of *Myrmica scabrinodis* in adult, pupal and larval stages in from one to six inches depth of soil. The colonies did not appear to have any disturbing effect on the rest of the population of the sample.

Two small adult individuals of the sub-family *Pteromalinae* (Chalcidae) were found beneath the surface; it has not been possible to identify them.

Specimens of *Aphidius danci* were taken from the soil as tiny cocoon-like structures; the species is a typical parasitic form which undergoes metamorphosis within the dead body of the host—a small Aphid—under which conditions it does not form a cocoon-like pupal covering. Here, however, the specimens were free in the soil and not in any intimate relation with a host. Other species of this genus are known to feed on the roots of plants.

With the exception of the *Collembola*, the *Acarina*(14) contributed the greatest number of individuals towards the soil fauna. These were distributed among four families and, excluding the nymphs, among 17 species.

The organisms occurred at all depths in the soil, but were most common in the surface three inches. They were ubiquitous, being found among the roots of the vegetation, free in the soil and on other soil animals. They appeared to respond to moisture conditions in much the same way as did the *Collembola*. In similar laboratory experiments, it was found that the Oribatid *Archipteria redux* offered the longest resistance to drought conditions, though this did not amount to more than four days in soil with initial water content 36 per cent. and which was allowed to dry at ordinary room temperature.

A. redux appeared to be a typical member of the permanent soil fauna of the field, and itself contributed approximately 77 per cent. of the total Acarine population. It was an extremely common species, occurring throughout the investigation although it is usually characteristic of fairly damp places. They were essentially vegetarian, feeding mainly on the moss amongst the roots and shoots of which they were most prevalent.

The most curious form was the vermilion sac-like and sluggish *Enemothrombium subrasum*, a form usually carnivorous with a preference for *Collembola* and *Tyroglyphidae*. The more usual habitats for *Thrombidiidae* are very damp places or even water.

Archipteria fimetorium typically breeds in manure heaps and is often found on members of the *Carabidae*. It was not infrequently met with in the investigation and there may be some connection with the fact that the field is naturally manured.

Four species of *Spiders* were recorded but were not studied in any detail as it is doubtful whether they were true soil inhabitants, although their eggs may be laid underground and the adults themselves were found among the looser upper fragments of soil by the bases of grass tufts.

Members of each of the three divisions of *Myriapoda* were present, but never in large numbers, and the variety in species was not great. Members of the *Symphyla* occurred most frequently and were distributed throughout the nine inches of soil, the numbers increasing with increase in depth. The individuals have not all been separately and specifically identified, but they appeared to be mainly of the same type, and included the species *Scolopendrellopsis subnuda*, *Symphylella delicatula* and *S. vulgaris*. They were relatively inactive and not very responsive to external stimuli and so far as could be ascertained were exclusively humus feeders. They contributed 86 per cent. of the total Myriapod population. Of the remaining divisions the Centipede *Geophilus longicornis* was the most common, although even this was only occasional.

The *Oligochaeta* usually form a very important section of the soil fauna and were of frequent occurrence during the earlier part of the investigation.

After January 1921 the numbers dropped very considerably. It is possible that the water content of the soil may have some bearing on this fact, but it does not afford a complete explanation.

For convenience an arbitrary division of the forms has been adopted: (i) larger Oligochaetes (*e.g.* Terricolae), (ii) small white Oligochaetes (*e.g.* Enchytraeidae); the members belonging to the latter have not been specifically named.

By far the greater number occurred in the first three inches of soil and it is mainly the larger forms which persisted in the lower depths. In a total of 830 approximately 76 per cent. was contributed by the "small white Oligochaetes" which were almost invariably found entwined round the rootlets of any type of plant. These forms varied in size from one-eighth to three-quarters of an inch in length, a variation which held for every sample in which they occurred. A great number were species of the red-blooded *Enchytraeids*, typically vegetable feeders, rather inert unless disturbed and rarely moving from the food supply. An indication of the type of species in the soil is given by the following list: *Fredericia unibaelsenia*, *F. bisetosa*, *F. diabeta*, *F. perrieri* and the Enchytraeid *Enchytraeus albidus*. Under drought conditions the majority appeared to shrivel and die off.

The "larger Oligochaetes" occurred fairly regularly in the form of the common species *Lumbricus terrestris* in all stages of development, and throughout the nine inches of soil, though predominantly in the upper three inches. The disappearance of this species during the dry summer months 1921 was possibly due to withdrawal to a lower depth than that with which the samples dealt. *Helodrilus oculatus* may be mentioned as being the first record for this species in Wales. It is interesting in being the only Lumbricid with eyespots, which are present for a short time during immaturity.

Several specimens were examined for parasites, but except for the common parasites *Monocystis agilis* and Nematodes in the intracellular portion of nephridia, no foreign bodies were evidenced beyond thick walled fungal resting spores and apparently harmless bacteria. Very occasionally, in the spermathecae, structures were present which were probably Nematode eggs.

No parasites were noted in the small white Oligochaetes so far as examined.

As in the Oligochaetes, the *Nematoda* have been for convenience artificially divided into two groups: (i) the large opaque white forms, (ii) the small colourless transparent forms. Again the greater numbers occurred in the upper three inches of soil, mainly due to the Nematodes which were prevalent during the months June to November, inclusive, 1920; after that date with three exceptions (totals 1, 5 and 1 respectively) they did not re-occur until the end of August 1921. This lapse of time seems rather too long to be due entirely to life cycles.

It is doubtful whether the given census figures are truly indicative of the Nematode population, as they are readily overlooked by reason of their smallness and transparency; they are best detected by floating upon water after sieving the soil, where they betray themselves by wriggling violently.

Many of the forms were Tylenchus-like in appearance, and the majority appeared to be free living in the soil, though there were examples of certain forms living in bulbs, on the bases of grass shoots, while others again parasitised certain of the fauna, as in the case previously recorded for the Collembolan.

The members of the improvised division "larger Nematodes" were found free living in the soil and slightly increased in numbers with increase in depth; typically they were beneath the root level and did not appear to be parasitic in any way, mainly feeding on humus. The forms chiefly belonged to the family *Mermithidae* (e.g. *Mermis brevis*).

Mollusca were very poorly represented and seem to have little importance in the general life of the soil fauna.

So far as could be ascertained there was no definite relationship between the fungi and the vegetation, and very little with the fauna of the soil. The only instances noted were attacks on injured organisms in both cases.

In connection with the fauna, fungal growth was found on beetles and earthworms and on pupae should the soil become waterlogged for more than a very short period. With regard to injured earthworms, it is probable that the attack is not wholly external, but rather due to the growth of the spores near the wound, which are inevitably in the alimentary canal by reason of the soil diet. An injured earthworm was found with a tuft of fungal hyphae covering the wound. Inoculations were made from this into beerwort agar and, after incubation, cultures of the following species were obtained: *Mucor racemosus*, *Absidia spinosa*, *Saccharomyces* sp., *Cladosporium epiphyllum*.

One beetle was captured, *Bradycellus verbasci*, with several strands of hyphae projecting from the head, and from between the abdominal segments. The fungus probably belonged to the genus *Monilia*.

4. COMPARISONS WITH OTHER AREAS INVESTIGATED.

(1) UNGRAZED GRASSLAND. The area now to be discussed has not been studied from the point of view of the seasonal census as such, but rather so as to form a comparison with the pasture land.

August, 1920

	Pasture field		Ungrazed grassland		May, 1920
Group	No. of spp.	No. of individuals	No. of spp.	No. of individuals	No. of individuals
Nematoda	1 +	69	1 +	64	7
Oligochaeta	10 +	86	10 +	326	563
Acarina	5	107	6	70	288
Araneae	—	—	1	1	—
Myriapoda	5 +	4	8 +	40	29
Collembola	8	66	6	757	803
Coleoptera	2	2	9	13	13
Diptera	2	7	1	1	8
Lepidoptera	—	—	—	—	1
Mollusca	—	—	1	2	4
Totals	33 +	341	43 +	1274	1716

The area is a piece of grassland where the vegetation, save for being occasionally scythed, was entirely undisturbed. It is separated from the pasture field by a hawthorn hedge raised on a bank. The two fields are essentially similar in physical conditions, the nature of the soil, and the vegetation.

A standard sample was taken on August 25th, 1920, both from the pasture field and from the ungrazed area: the first four columns of the preceding list give some idea of the relative frequency of the species and individuals present.

The population of the ungrazed area shows a marked increase over that of the pasture field, both in number of species and in number of individuals. An analysis of the table shows that as regards individuals this increase is in the main due to the *Collembola* and *Oligochaeta* and to the *Myriapoda* to a lesser extent (see Fig. 5). The *Acarina* is the only group where there is any very perceptible decrease in the ungrazed area, but it is doubtful whether any significance should be attached to this, in view of the high numbers recorded for a sample previously taken in May.

The greater numbers were probably due to three main reasons:

(i) Effective shelter and protection for insects in the long grass—from high winds and enemies.

(ii) The soil was kept in a cool, moist condition for a longer time because of the long grass.

(iii) Trampling by the larger grazing animals causes the soil to become compressed into a compact layer, causing diminution of air spaces and rendering efficient respiration more difficult. The soil of the ungrazed area is thus naturally more "open" than the pasture field.

The relative distribution of the fauna in depth did not materially differ from that of the pasture field, being concentrated in the upper three inches.

The dominant species in both areas were markedly the Collembolans *Onychiurus ambulans, O. armatus*, and the Acarine, *Archipteria redux.*

The following list gives the species which occurred in the ungrazed area, but not in the pasture field.

MYRIAPODA: ***Brachydesmus superus*** var. ***mosellans, Schlendyla memorensis, Banjulus*** sp., ***Polydesmus collaris.***

COLLEMBOLA: ***Megalothorax minimus, Bourletiella hortensis*** var. ***juvenilis.***

COLEOPTERA: ***Melolonthus vulgaris, Syntonium*** sp., ***Bembidium obtusum, Polystethus*** sp., ***Enicmus minutus, E. transversus, Tachyporus chrysomelinus, Apion virens.***

DIPTERA: ***Drymeia hamata.***

A comparison between samples taken from the ungrazed area in May and August 1920 shows that the total soil population was markedly greater in May (see last column in the previous table). Also the total population in either month exceeded any total per sample recorded for any other area which has been examined.

The greater numbers in May were due to contributions from *Acarina* and *Oligochaeta*; on the other hand, *Myriapoda* and *Nematoda* showed higher totals for August.

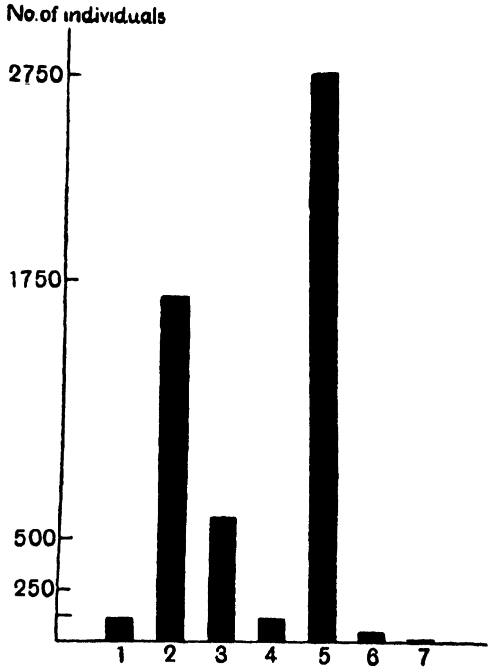

Fig. 5. *Ungrazed Land.* Relative frequency of occurrence of individual groups. Scale—1 cm. = 250 individuals.

1, Nematoda; 2, Oligochaeta; 3, Acarina; 4, Myriapoda; 5, Collembola; 6, Coleoptera; 7, Diptera.

Totals of other groups too small to be included in diagram.

Cast skins of Collembolans were abundant in each month, though varying in distribution, 31 skins being found in the upper three inches and none elsewhere in May, whereas in August one only was found in the upper three inches and 31 in the 6–9 inch layer. These facts are probably in the first place due to changes in the water content of the soil, *i.e.* in August when the soil is normally much drier than in May, the organisms would probably migrate to lower depths for the purpose of casting their skins, where the water content would be somewhat higher, and there would be less danger of desiccation at a critical period.

One case was noted of parasitism by Nematodes. *Megalothorax minimus* (*Neelidae*) may be mentioned as the only record of this species for the whole investigation; as the form is microscopic it is possibly more prevalent than the numbers appear to indicate.

Species usually associated with dung or refuse were occasional; these may or may not be invaders of the area. They were the Coleopterans *Megasternum boletophagum*, *Enicmus minutus* and *E. transversus*.

(2) LAND RECENTLY PLOUGHED. This adjoined the ungrazed grassland, and was essentially similar in general physical conditions and the nature of the soil.

A standard sample was taken four months after the layer of turf had been removed from the area, and a few days after hoeing. The soil was in a loose open condition and approximated in this respect to the soil of the cultivated land, to be discussed later.

From the treatment of the area four results were inevitable:

(i) That the majority of the organisms should be removed with the turf layer.
(ii) That with the removal of the turf layer the main source of food should also be removed.
(iii) That a certain amount of direct injury would be done to the fauna by ploughing and hoeing.
(iv) That temporary conditions of drought would be set up for a time in the surface layers of soil.

The analysis of the sample is given in the first column of the table on page 380.

Dealing with the May sample, the first noticeable fact was the paucity of the fauna, with the exception of the *Collembola*.

Another marked feature was that the population of the first three inches of soil was *less* than that of the lower levels, the numbers increasing with depth.

In the Collembolan group only *Achorutids* were present; *Coleoptera*, *Diptera*, *Acarina* and *Myriapoda* were very poorly represented and Nematodes were absent. The Chilopod, *Lithobius forficatus*, was the only species recorded in the ungrazed grassland.

After the above examination, weeds were allowed to grow freely for

		1920	
Group		May	July
Nematoda	Nematodes	—	4
	Long opaque forms	—	1
Oligochaeta	Small white forms	7	25
	Lumbricus terrestris	1	12
	Helodrilus oculatus	—	7
	Allolobophora chlorotica	—	1
Myriapoda	Symphyla	2	20
	Brachygeophilus truncorum	—	1
	Geophilus longicornis	2	2
	Lithobius forficatus	—	3
Acarina	Gamasus fimetorium	1	2
	Pergamasus crassipes	1	4
	P. runcatellus	—	2
	P. meridionalis	—	1
	Nymphs	1	—
	Archipteria redux	—	6
Collembola	Onychiurus ambulans	93	23
	O. armatus	76	26
	O. fimetarius	93	23
	Entomobrya muscorum	—	1
	Isotoma viridis	—	16
	Isotomurus palustris	—	9
	Beckia argentea	—	3
	Sminthurus viridis	—	1
Coleoptera	Amara communis	—	1 A
	Nebria brevicollis	—	3 L
	Xantholinus linearis	—	6 A
	Polystethus sp.	—	2 L
	Agriotes obscurus	3 L	1 L
	Weevil sp.	3 L	1 L
Diptera	Trichocera hiemalis	—	1 L
	Tipula sp.	1 L	—
	Psila rosea	—	1 L
Hymenoptera	Myrmica scabrinodis	19	—

two months, when a further sample was taken. By this date the surface was fairly closely covered by a mixture of the following plants:

A few *Poa* grasses, *Dactylis glomerata*, *Euphrasia officinalis*, *Lapsana communis*, *Hypochoeris radicata*, *Fumaria officinalis*, *Achillea millefolium*, *Anagallis arvensis*, *Plantago lanceolata*, *Trifolium repens*, *Bellis perennis*, *Viola tricolor*, *Geranium bisectum*, *Cerastium vulgaris*.

With the appearance of this covering of vegetation the majority of the fauna became intimately connected with the roots, *i.e.* the population became once again by far the greatest in the upper three inches, decreasing with depth. There was, moreover, a substantial increase in variety of species. So that in density, variety and vertical distribution there was some approach to the pasture land. The totals for the July sample are included in the above table.

Curiously, the *Collembola* showed a fall in total numbers, though the variety in species increased; the dominant form was *Onychiurus armatus*.

The *Achorutidae* generally were extremely small and Nematode attack was noted.

Among the other groups several species occurred which were not recorded for the ungrazed grassland or pasture, viz.: *Trichocera hiemalis* and *Psila rosea* among the *Diptera* and a *Scarabaeid* larva among the *Coleoptera.*

(3) CULTIVATED LAND. The land is about half the area of the pasture field and is situated immediately below the National Library on a steep slope about 4° from the horizontal (Fig. 1). To the N. other arable land adjoins it, while the remaining sides are bounded by more or less waste land. The position is sheltered to some extent from all but W.S.W. winds.

The primary purpose of obtaining samples from this area was for comparison with the pasture land. The seasonal cycle was however followed through six winter months.

Samples were taken from opposite ends of the area: (i) from a part with rows of *Trifolium,* (ii) from a portion with rows of *Perennial Rye grass.* Both these parts were kept carefully weeded.

Three years before the investigation, the ground was used for potato growing, being more heavily manured in the present clover section. The following year it was again manured and since then has not been touched. Early in 1920 the rye grass section was treated with a surface dressing of basic slag (10 cwts. to the acre). This difference in treatment has not, so far, led to any marked difference in the type of fauna occurring in the two parts, so that the organisms from these areas will not be discussed separately, with one notable exception in the group *Myriapoda.*

As was the case for the pasture area, the soil is open and porous in nature and the substratum shaley, so that there is no permanent waterlogging; the slope of the land also tends to promote rapid drainage. The *water content* estimations showed no marked difference from the pasture field though they were often slightly higher—in one case as much as 7 per cent. This is possibly associated with the rather larger proportion of humus in the cultivated land. The figures for the weekly water content estimations of "representative" samples varied in a manner similar to those of the pasture area and are shown graphically in Fig. 6 in which is plotted also the census for the fauna of the arable land. The population curve of the pasture field falls lower and rises higher than that for this area. Fig. 7 shows the relative frequency of occurrence of the individual groups.

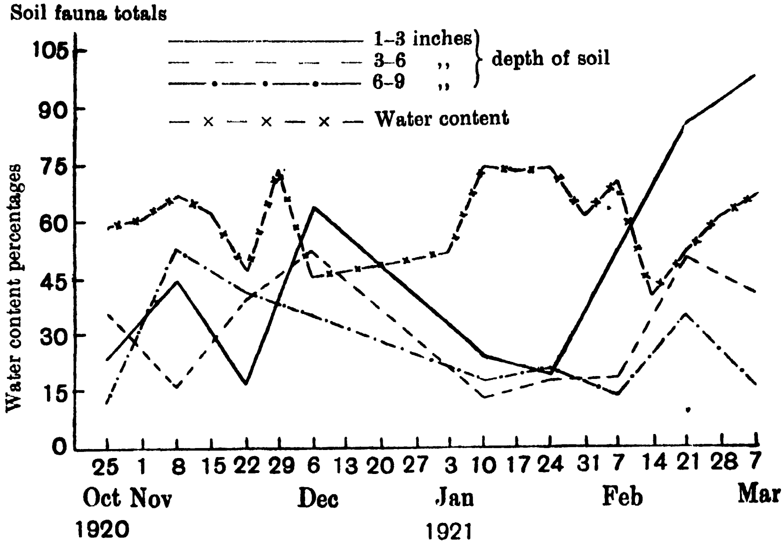

Fig. 6. Cultivated land: total fauna curves.

DISTRIBUTION OF THE SOIL FAUNA OF ARABLE LAND*.

[2 samples per month]
Oct. 1920–Mar. 1921

NEMATODA			
Nematoda spp.	A 2 10, 11	:	B 1 11
Large white forms spp.	A 3 : 2 (1) 11, 1	:	B 3 10
OLIGOCHAETA			
Small white forms spp.	A 20 : 5 (11) 10, 11, 12, 1, 2	:	B 52 : 8 (10) 10, etc. to 3
Lumbricus terrestris	A 4 : 2 (3) 2, 3	:	B 1 12
Helodrilus oculatus	A 1 10	:	—
Allolobophora chlorotica	A 1 1	:	B 1 11
ARACHNIDA			
(1) Gamasidae			
Pergamasus crassipes	A 5 : 2 (3) 12, 1, 2, 3	:	B 3 : 1 11, 1
P. septentrionalis	A 1 1	:	B 3 11, 12, 2
Oligogamasus pollicipatus	A 1 11	:	—
Gamasus fimetorium	—	:	B 1 3
G. magnus	A 1 11	:	—

	[2 samples per month] Oct. 1920-Mar. 1921		
ARACHNIDA (*contd.*)			
Pachylaelops sp.	A 1 1	:	—
Spp. Nymphs	A 2 12, 3	:	B 1 11 —
(2) Thrombidiidae			
Bdella lignicola	A 2 11, 3	:	—
(3) Oribatidae			
Archipteria redux	A 7 : 2 (1, 2, 3) 12, 1, 2, 3	:	—
MYRIAPODA			
(*a*) *Symphyla*			
Spp.	A 9 : 5 (11) 10, 11, 12	:	B 8 : 3 (10) 10, 11, 2
(*b*) *Diplopoda*			
Polydesmus collaris	A 11 : 6 (11) 11, 12, 1, 2	:	B 30 : 8 (11) 11, 12, 1, 2, 3
(*c*) *Chilopoda*			
Geophilus longicornis	A 4 11, 12, 1, 2	:	B 13 : 6 (12) 11, 12, 1
Schlendyla memorensis	A 2 10	:	B 1 2
Brachygeophilus truncorum	A 2 1, 2	:	B 3 : 2 (12) 12, 1
Lithobius forficatus	A 6 : 4 (12) 11, 12, 1		B 35 : 17 (11) 11, 12, 1, 2, 3
INSECTA			
(*a*) *Collembola*			
(1) Achorutidae			
Onychiurus ambulans	A 133 : 73 (3) 10, etc. to 3	:	B 126 : 24 (3) 10, etc. to 3
O. armatus	A 128 : 49 (2) 10, etc. to 3	:	B 87 : 24 (2) 10, etc. to 3
O. fimetarius	A 24 : 10 (2) 11, 12, 1, 2	:	B 76 : 13 (11) 10, etc. to 3
(2) Entomobryidae			
Entomobrya muscorum	A 6 : 3 (11) 10, 11, 1	:	B 3 : 2 (10) 10, 2
E. multifasciata	A 14 11, 3	:	B 19 : 17 (2) 2, 3
E. Nicoleti	A 2 11	:	—
Isotoma viridis	A 5 : 3 (2) 10, 11, 2	:	—
I. plumbea	A 1 2	:	—
Isotomurus palustris	A 3 10	:	B 1 11
Folsomia fimetaria	A 11 : 10 (2) 2, 3	:	B 19 : 10 (3) 2, 3

[2 samples per month]
Oct. 1920–Mar. 1921

INSECTA (*contd.*)			
Beckia argentea	—	:	$\frac{B\ 4:1}{11, 1}$
(3) Sminthuridae			
Sminthurus luteus	$\frac{A\ 1}{2}$	:	—
(*b*) *Coleoptera*			
(1) Carabidae			
Nebria brevicollis (L)	$\frac{A\ 11:3\ (11, 12, 2)}{11, 12, 1, 2}$	:	$\frac{B\ 5:1}{11, 12, 1}$
(2) Staphylinidae			
Xantholinus linearis (A)	—	:	$\frac{B\ 2}{1, 2}$
Philonthus sp. (L)	—	:	$\frac{B\ 3}{11, 1, 2}$
Sp.	—	:	$\frac{B\ 1}{1}$
(3) Pselaphidae			
Euplectus kunzei (A)	—	:	$\frac{B\ 1}{2}$
(4) Scarabaeidae			
Aphodius fimetarius (A)	$\frac{A\ 1}{12}$	:	—
(5) Elateridae			
Agriotes obscurus (L)	$\frac{A\ 7}{12}$	:	$\frac{B\ 2}{10, 12}$
(*c*) *Diptera*			
(1) Tipulidae			
Tipula paludosa (L)	—	:	$\frac{B\ 1}{12}$
(2) Leptidae			
Leptis scolopacea	$\frac{A\ 1}{1}$	:	—
L. tringaria (L)	—	:	$\frac{B\ 1}{1}$
(3) Brachycera			
Sp. (L)	—	:	$\frac{B\ 1}{11}$
(*d*) *Hymenoptera*			
Aphidius danci (C)	$\frac{A\ 1}{3}$	:	—

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* A formula similar to that previously used is employed above. The maximum number of individuals for any one sample is here given for B as well as for A, owing to the relatively greater concentration of numbers in the lower layers compared with that in pasture land.

The *maximum water content*, *pore space*, *hygroscopic water*, were all estimated from a similar number of samples to those used in the case of

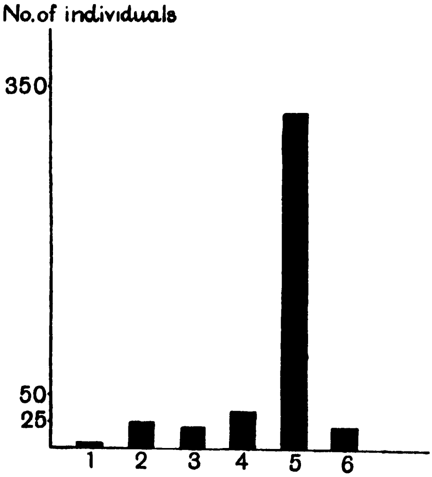

Fig. 7. *Cultivated Land.* Relative frequency of occurrence of individual groups. Scale—1 cm. = 50 individuals.

1, Nematoda; 2, Oligochaeta; 3, Acarina; 4, Myriapoda; 5, Collembola; 6, Coleoptera.

Totals of other groups too small to be included in diagram.

the pasture field. In each case the figures were rather higher for the arable land. The humus content was lower however.

	Average percentage in	
	i. Arable land	ii. Pasture land
Maximum water content	84·25	81·56
Pore space	57·50	48·93
Hygroscopic water	3·70	3·45
Humus content	10·73	12·87

Whilst the temperature readings were generally similar to those of the pasture, the more sheltered position of the arable land was clearly shown by the records; in most cases the temperature of both the atmosphere and the soil was slightly higher than the corresponding readings for the pasture land.

Mechanical analysis of the soil of this area shows that it differs little from that of the pasture field in the majority of its components; the most notable points of difference are the greater percentage of coarse sand and the much reduced percentage of fine gravel. Stones of all sizes are much more numerous.

Standard samples of soil were taken every fortnight during the winter months and the faunistic results obtained (see tables) are collected in the previous table.

(i) As in the previous areas the forms present belonged to the groups *Nematoda, Oligochaeta, Myriapoda, Isopoda, Arachnida, Insecta.*

(ii) Both in number of species and of individuals all the groups which occurred are less than in the pasture area. In the pasture tables all the species of the arable land are included with the exception of the Chilopod *Lithobius forficatus.* The fact that the fauna occurred in such small numbers was probably due mainly to the lack of a turf covering and therefore of food, also to a lesser extent to the fairly frequent hoeing and weeding, resulting in a certain amount of direct injury also indirect through desiccation of the soil. These conditions were similar to those for the land recently ploughed.

(iii) The very small number of *Acarina* present may be particularly referred to.

(iv) The tables give the distribution in depth, the facts of which are shown clearly by the curves in Fig. 6. On comparison of the latter with the corresponding pasture land curves (Fig. 2), the outstanding feature is that the curves for the arable land show that the population of the upper three inches hardly exceeds that at a depth of from 3 to 6 inches or from 6 to 9 inches. A notable exception occurred on March 7th, where the totals for the upper and middle three inches had risen markedly above that for 6 to 9 inches which had fallen, but even here the middle curve closely follows the upper. In three samples the numbers which occurred in one or both of these lower layers even exceeded those in the first three inches. This is comparable with conditions in the recently ploughed area.

(v) Of the *Insecta* the *Collembola* were ubiquitous and the best represented in the area, though the variation in species was not so wide as in the pasture field; the same species were dominant in both, viz.: *Onychiurus ambulans* and *O. armatus* which together contributed 72 per cent., approximately, of the total Collembolan population. Young forms were found fairly evenly distributed throughout the samples, but all sufficiently developed for identification.

Cast skins occurred very frequently at all depths and in all samples.

The individuals throughout the samples were mainly from the *Achorutidae.* A greater proportion than usual of *Entomobryidae* occurred below three inches depth, probably due to the loose nature of the soil.

(vi) Among the *Oligochaeta* the small white forms alone occurred with any constancy; usually they were found coiled beneath small flat stones and fairly evenly distributed throughout the nine inches depth of the sample.

Points of lesser interest were:

(i) The occurrence of large numbers of *Myriapoda* in the samples taken November 22nd and December 6th. Individual plants of Perennial Rye grass had been planted a short time before, and a complaint made to the effect that the plants were dying off. On examination of a sample taken from the affected area on November 22nd, the trouble was found to be due to numbers of the small Diplopod *Polydesmus collaris*, of which 22 were found in one sample, rapidly eating away the roots of the plants in question. The Chilopod *Lithobius forficatus* was also present in similar numbers, a very active small carnivorous organism.

A fortnight later, the Rye plants were no longer dying off and the numbers of *Polydesmus* were found reduced to an average of ten per sample and *Lithobius* to seven. Besides the reduction in numbers of the injurious species, the plants had probably completed the critical unsettled period after planting out. *Polydesmus* did not occur before or after this particular period.

The *Symphyla* were less common than in the pasture field.

(ii) The Hymenopteran *Aphidus danci* occurred in the same unusual manner as in the pasture field, the pupa being free in the soil instead of living within a host.

5. ACKNOWLEDGEMENTS.

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6. NOTES ON PREVIOUS PAPERS AND SOME COMPARISONS.

The first intensive record concerning soil insects in relation to their environmental conditions, was published by Cameron (4). In that investigation and also in the present one, fields of permanent grassland were examined, though the Manchester area offered less uniform conditions affording several sub-habitats and included rotting trunks and vegetable refuse. Again the vegetation was much more heterogeneous than in Aberystwyth, in particular the variety of weeds. So that a qualitative comparison is hardly possible. A quantitative comparison is also impossible as Cameron did not work with standard samples.

The richer fauna in the Manchester field is probably due in part to the entering of species from the varied surroundings. The small number of *Collembola* found is probably due to the method of examining the soil; the smaller forms are difficult to distinguish unless the soil is sieved and the organisms floated upon the surface of water.

Cameron found forms occurring to a depth of 12 inches, while in Aberystwyth they rarely occurred below nine inches. In the case of the *Collembola*, however, while the Manchester forms were limited to the surface and first three inches, the Aberystwyth *Achorutidae*, at least, occurred frequently to a depth of nine inches or even below.

In both areas the roots *Poa pratensis* and *P. annua* formed food for similar Dipterous larval forms representing the families *Mycetophilidae*, *Bibionidae* and *Tipulidae*.

The second paper by Cameron (5) dealt with a similar investigation in Cheshire. It included many types of land from which "Glover's Meadow" is selected for a rough comparison; this is composed mainly of clay loam.

Only eight species are common both to this field and to the Aberystwyth area, out of approximately 58 and 85 species respectively.

A paper by Morris (19) forms an interesting and much closer comparison as he worked with a more limited area by means of standard nine-inch cube samples which is the size adopted by the present writer. Both areas have been in pasture for about 40 years, have a similar vegetation and are approximately the same height above sea level. Geologically, the Cheshire district presents a younger formation than Aberystwyth, though boulder clay overlies both. Mechanical analyses show very considerable differences between the types of soil; in Cheshire, the percentages of silt, coarse sand and fine sand being markedly greater and the other components considerably lower than those for Aberystwyth. In the Cheshire field a clay substratum is reached at about a foot deep and very little humus occurs below six inches, while at Aberystwyth the soil seems to be of the same nature for a considerable depth, probably until the shale rocks themselves are reached, and the organic content is as great or greater from six to nine inches as from one to six inches.

On consideration of the data recorded for both areas, the following points are evident:

(i) In both areas, the vast majority of organisms occur in the top two or three inches, particularly so in Cheshire.

(ii) That with the exception of the *Collembola* and *Diptera* there is no great difference in the total numbers of species recorded in the two investigations. In the case of both these orders the number of species is more than twice as great in the Aberystwyth soil and the number of individuals is much greater.

(iii) That while the composition of the insect fauna of the soil in the two cases is mainly due to representatives of *Collembola*, *Coleoptera* and *Diptera*, the relative contribution of the orders expressed in species differs a good deal in the two cases. Of the total number of species represented by these three orders the *Collembola* contribute 30 per cent., the *Coleoptera* 46 per cent. and the *Diptera* 24 per cent. at Aberystwyth, the corresponding percentages for Cheshire being 19, 67 and 14. Few only of the species are common to the two investigations.

(iv) That while in each case a relatively small number of the species present provides the great majority of individuals, the dominant species in the two regions are not generally the same.

A second paper by Morris (20) deals with cultivated land and a comparison may be instituted with the cultivated land of the present paper.

The land of the two areas differs considerably in situation, nature of the soil and weeds—particularly the latter, which apparently are constantly present in the Rothamsted area, while the Aberystwyth cultivated land is kept carefully weeded.

In neither case did such a high percentage of the population occur in the first three inches as in grassland, but whereas Morris found the population of the first three inches markedly greater than the lower levels, in Aberystwyth it was only slightly in excess of the layers beneath. Perhaps this may be associated with the difference in quantity of weeds.

Morris deals in both manured and non-manured plots, and in both cases the variation in species is invariably richer than Aberystwyth. Very few of the species are common to both areas:

COLLEMBOLA: *Onychiurus fimetarius*, *O. ambulans*, *Isotoma viridis*, *Isotomurus palustris*, *Entomobrya multifasciata*.

MYRIAPODA: *Geophilus longicornis*.

ACARINA: *Gamasus magnus*, *Pergamasus crassipes*, *P. septentrionalis*.

A recently published paper by Buckle (3) concerns itself primarily with the effects of cultivation, but includes a Lancashire pasture field as a control. Comparing the latter with the Aberystwyth pasture one notes that the environmental conditions, geological formation of the land and the mechanical nature of the soil differ widely in the two areas. Buckle adopts the 9-inch cube as his standard sample.

Summarising the table for the "Big Meadow" and comparing this with the Aberystwyth data, one arrives at the table below, showing the number of species in the two cases (the figures in brackets indicate the number of individuals).

October to May inclusive.

	Lancashire pasture 1919–20 9″ cube of soil	Aberystwyth pasture	
		1920–1 9″ cube of soil	1921–2 1–3″ depth
Acarina	—	15 (1149)	13 (2079)
Chilopoda	3 (3)	2 (13)	1 (4)
Diplopoda	—	1 (3)	1 (1)
Symphyla	—	? (71)	? (30)
Collembola	—	20 (1453)	18 (5292)
Rhynchota	—	2 (3)	2 (4)
Coleoptera	18 (29)	20 (95)	20 (115)
Lepidoptera	—	—	3 (3)
Diptera	6 (21)	16 (248)	12 (136)
Hymenoptera	1 (1)	1 (1)	—
Totals	28 (54)	77 (3036)	70 (7664)

Buckle's method of investigation involved careful sieving, but no floating out of small forms on water was undertaken. This difference in methods may explain the absence of *Collembola*, *Acarina* and *Symphyla* from Buckle's records and their presence in Aberystwyth samples, where the two former orders at least are the dominant forms.

In the same paper Buckle draws the following conclusions:

(i) That distribution and numbers are more stable in the fauna of grassland than of arable land.

(ii) That there is a seasonal increase in the fauna of *grassland* as the growth of vegetation increases.

(iii) That there is a seasonal increase in the fauna of *arable* land as the growth of vegetation increases.

The Aberystwyth data amply support No. (i) above, but more doubtfully (ii) and (iii).

The total fauna numbers increase markedly in early winter for Aberystwyth while Buckle says that his increase takes place with the growth of vegetation. But his orders only include *Chilopoda, Coleoptera, Diptera* and *Hymenoptera,* while the main orders determining the Aberystwyth curve are the *Collembola* and *Acarina.*

If analysis be restricted to the dominant groups of Buckle, viz. *Coleoptera* and *Diptera,* then as in Lancashire there is certainly an increase in Spring, but in addition there is another increase in early Winter.

However, it seems dangerous to generalise from such small totals, particularly as the inclusion of colonies has a wide influence on the trend of the total fauna curve.

The highest numbers for Aberystwyth in the following tables (136, 52, 91) are due to colonies of Dipterous larvae.

Number of Individuals per month.

	June	July	Aug.	Sept.	Oct.	Nov.	Dec.	Jan.	Feb	Mar	Apr.	May
Aber. past., 1920–1	16	17	9	—	32	52	—	136	27	42	22	32
Aber. past., 1921–2	24	15	13	16	15	34	12	91	10	24	26	39
Aber. arable land, 1920–1 ...	—	—	—	—	3	7	15	9	6	0	—	—
Lancs. meadow, 1919–20 ...	—	—	—	—	0	0	0	4	11	7	17	11
Lancs. arable land, 1919–20	—	—	—	—	0	2	0	0	0	0	0	54
Lancs. "broken-up," 1919–20	—	—	—	—	5	0	0	1	3	1	11	31

7. SUMMARY.

1. An attempt has been made to give an account of a systematic qualitative and quantitative seasonal investigation of the macroscopic soil fauna of a limited area, and to correlate the data obtained with the general environmental conditions.

The piece of land most carefully investigated was a pasture field from which soil samples were obtained fortnightly throughout two successive years, but for purposes of comparison, observations were also made in connection with ungrazed grassland, similar ungrazed land with the turf recently removed and cultivated land; the soil of these areas is very similar in physical, chemical and geological characteristics.

2. The method of investigation included the taking of standard nine-inch cube samples. A preliminary sorting of the soil particles was followed by sieving the soil in water and placing the residue with water in a shallow dish, when the smaller organisms floated to the surface.

3. (*a*) The main area studied has been in pasture for over 40 years and has a typically mesophytic vegetation. Water content percentages were fairly high. The temperature of the soil varied with depth, being usually higher at 9 inches depth than at 4½ inches; it varied much less than the temperature of the atmosphere.

(*b*) With rare exceptions the population in the surface three inches was markedly greater than that in the lower depths, probably because the majority of soil organisms are root feeders and the average root level ends at about three inches depth; carnivorous forms would naturally tend to concentrate here also. Another reason may be found in the better aeration of the soil in the surface layers.

(*c*) The orders *Collembola* and *Acarina* together determine the trend of the total fauna curve, *i.e.* they are the markedly dominant groups of the soil population as regards the number of individuals. A very few species are in turn responsible for the trend of the *Collembolan* and *Acarine* curve (Fig. 3); the high total numbers of the population are mainly contributed by the Collembolans, *Onychiurus ambulans*, *O. armatus* and to a lesser extent by *Isotoma viridis* and *Folsomia fimetaria.*

(*d*) Some orders such as *Collembola* and *Acarina* persist in varying numbers throughout the year, while other groups such as *Oligochaeta* and *Nematoda* entirely or almost entirely fall out for varying lengths of time. In the persistent groups, the same species remain dominant throughout the year.

(*e*) The maximum population occurs in the winter months; in part probably due to the relative absence of drought conditions in the winter, water content being more constant. The two summers have low totals for the fauna. Water content presumably plays an important part in connection with the small white *Oligochaeta.* Some preliminary experiments were carried out with regard to the effect of drought on *Collembola* and *Acarina*; it was found that life was very much curtailed by drought conditions.

4. The fauna of the *ungrazed grassland area* was similar to the pasture field except that the population was much greater. The richer character of the fauna is probably due to the protection of the long grass and the prevention of undue evaporation of moisture from the soil; being ungrazed the soil is less compressed.

5. The examination of *land recently ploughed* resolved itself into a survey of the results following (i) a removal of the turf layer; (ii) the further result of allowing the land so treated to be untouched for a definite time. With the removal of the turf, also goes the main source of food supply together with the majority of the soil fauna, and temporary conditions of drought are set up in the surface layers of soil. The main points of interest were: (i) the scarcity of the fauna except the *Collembola*; (ii) before weeds were allowed to grow the population of the upper three inches was *less* than that of lower levels, the numbers increasing with depth; (iii) with the growth of weeds the fauna showed some approach once more in density, variety and distribution to the grassland population.

6. The examination of *cultivated land* gave results very similar to those obtained for the previous area. The population is considerably less than that of the pasture. Generally speaking, the population of the

surface three inches of soil hardly exceeds that at a depth of three to six or six to nine inches. The same orders occurred as in the other areas.

7. Organisms injurious to the vegetation were present in all areas, but not usually in sufficient numbers to cause any damage. An exception occurred in cultivated land in which the roots of Perennial Rye grass were eaten away by the Diplopod *Polydesmus collaris*.

8. Parasitism was infrequent but at least the *Achorutidae* were subject to attack by small Nematodes.

Fungi were not normally found parasitic on the soil fauna; such attack appeared to be restricted to injured specimens.

9. A brief review has been given of work previously published and comparisons made with the present paper.

BIBLIOGRAPHY.

(1) ADAMS, C. C. (1913). *Guide to the study of Animal Ecology.* New York.

(2) BRAUER, A. *Süsswasserfauna Deutschlands.* Heft 12, Acarina.

(3) BUCKLE, P. (1921). A Preliminary Survey of the Soil Fauna of Agricultural Land. *Annals App. Biol.* VIII, Nos. 3 and 4.

(4) CAMERON, A. E. (1913). General Survey of the Insect Fauna of the Soil. *Journ. Econ. Biol.* VIII, Pt 3.

(5) —— (1916). The Insect Association of a local Environmental Complex in the district of Holmes Chapel, Cheshire. *Trans. Roy. Soc. Edin.* LII, Pt 1 (No. 2).

(6) CLEMENTS, F. E. (1915). *Research Methods in Ecology.*

(7) COLLINGE, W. E. *Manual of Injurious Insects.*

(8) CURTIS, J. *Farm Insects.*

(9) DALE, MISS E. On the Fungi of the Soil. *Annales Mycologici,* x, No. 5, 1912; XII, No. 1, 1914.

(10) FORD, G. H. (1917). Larval and Papal Stages of Agriotes Obscurus. *Annals App. Biol.* Jan.

(11) FOWLER, W. W. (1899). *British Coleoptera.*

(12) HALL, A. D. (1904). Mechanical Analyses of Soils. *Trans. Chem. Soc.* LXXXV.

(13) HOLE, R. S. (1919). Soil Aeration. *Journ. Ecol.* Nos. 1 and 2.

(14) HULL, Rev. E. J. Terrestrial Acari of the Tyne Province. *Trans. Nat. Hist. North Durham and Newcastle-on-Tyne,* IV, Pt 2; V, Pt 1.

(15) JENSEN, C. N. (1912). Fungus Flora of the Soil. *Cornell Univ. Agric. Expt. Station, Bull.* 315, June.

(16) KEEN, B. A. (1920). The Relations existing between the Soil and its Water Content. *Journ. Agric. Sci.* X, Pt 1.

(17) LUBBOCK, Sir J. (1871). Monograph on Collembola and Thysanura. *Roy. Soc.*

(18) MALLOCH, J. R. (1917). Preliminary Classification of Diptera exclusive of Puparia, based upon Larval and Pupal Characters. *Bull. Illin. State Lab. Nat. Hist.* XII, Pt 3.

(19) MORRIS, H. M. (1920). Observations on the Insect Fauna of Permanent Pasture in Cheshire. *Annals App. Biol.* VII, Nos. 2 and 3.

(20) —— (1922). The Insect and other Invertebrate Fauna of Arable Land at Rothamsted. *Ibid.* IX, Nos. 3 and 4, Nov.

(21) Morris, H. M. (1921). Larval and Pupal Stages of the Bibionidae. *Bull. Entomol. Research*, xii, Pt 3, Nov.

(22) Perris, M. E. (1877). *Larves de Coléoptères*. Paris.

(23) Rabenhorst, I. *Kryptogamen-Flora*. Pilze 2.

(24) Roberts, A. W. Rymer (1919). On the Life History of "Wireworms" of the Genus *Agriotes* Esch., with some notes on that of *Athous Haemorrhoidalis* F. *Annals App. Biol.* vi, Nos. 1 and 3.

(25) Sharp, D. (1910). Insects, Myriapoda, Worms. *Camb. Nat. Hist.*

(26) Shelford, V. E. (1912). Animal Communities in South America. *Geog. Soc. Chicago.*

(27) Theobald, F. V. (1920). *Report on Econ. Zool.* Sept.

(28) —— (1892.) *An Account of British Flies.*

(29) Verrall, G. H. (1901 and 1909). *British Flies.* 2 vols.

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THE LIFE-HISTORY AND HABITS OF *TORTRIX PRONUBANA*, HB., WITH SPECIAL REFERENCE TO THE LARVAL AND PUPAL STAGES

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(With Plate XV and 16 Text-figures.)

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Classification.

Order LEPIDOPTERA.
Group TORTRICINA.
Family *Tortricidae.*

I. INTRODUCTORY.

Tortrix pronubana is first recorded as occurring in Britain in October, 1905, when a single specimen of the moth was taken at Eastbourne (Adkin, 1905). It is recorded as having appeared in Guernsey in 1898 (Lowe, 1900). The moth is of Continental origin and was figured, without description, by Hübner in 1797 and 1830. According to Millière (1859), the species is a native of Southern Europe, Sicily, Corsica, Italy and Spain. Since its first appearance in Britain in 1905—it is evident that the moth has firmly established itself in the South of England—gradually working its way North. Within the past three or four years the species has become so abundant in the South as to prove harmful. In glass-houses at the Royal Botanic Gardens, Kew, serious damage is being done by the larvae to a great variety of plants.

The general life-history of the insect has been worked out by Adkin (1906 and 1908), who, however, has not given a detailed description of the larval stages. Accordingly, it was decided to conduct a further and more detailed investigation into its life-history and habits—with special reference to the larval and pupal stages. Material for the investigation was obtained from the Royal Botanic Gardens, Kew, and the insect reared in the laboratory on carnation plants and privet cuttings. Carnations have been badly attacked by this insect in France (Molinas, 1914). The laboratory experiments, conducted in the Entomological Laboratory of the Forestry Commission at Kew, under the supervision of Dr J. W. Munro, and also in Dr R. Stewart MacDougall's Laboratory in the University of Edinburgh, were amplified and supplemented by observations made in the glass-houses at Kew.

Tortrix pronubana is an interesting example of an insect which has within recent years appeared in our country from the South, gradually establishing itself and spreading northwards as it adapts itself to its new environment and climatic conditions. At the present time its actual distribution in Britain is not known but it would appear that, in the London district, where the work was carried out, the moth is present in large numbers, in the open, south of the Thames—while north of the river, although to be found, it does not occur abundantly. Continental references to *T. pronubana* state that the larva is polyphagous. This is amply borne out under glass at Kew, but in the open the larvae are found abundantly, chiefly on privet, euonymus and ivy.

II. LIFE-HISTORY AND DESCRIPTION OF STAGES.

THE MOTH.

Description.

The following is a description of the moth given by Adkin (1907) and which appears to be the only one which has been published in Britain:

"*Tortrix pronubana* is easily recognised by the bright orange colour of its hind wings, which at once distinguishes it from all its allies that are known to occur in Britain.

"*The Male* measures from 15–17 mm. in expanse. Fore wings warm greyish brown, faintly reticulated with darker brown, with a broad rich red brown fascia from the middle of the costa, where it is narrowest, to the inner margin, where it broadens out, extends to the anal angle and unites with an irregular triangular patch of the same colour that occupies the apical and hind marginal areas. There is also a dark shade on the inner margin between the fascia and the base of the wing. The reticulations on the darker markings have a distinct bluish colour when seen in a bright light. Hind wings bright orange, bordered with black, with a few black scales scattered along the veins. Cilia of the fore wings, brownish orange, of the hind wings pale orange.

"*The Female* is a larger and more sombre insect. It measures from 18–22 mm. in expanse, is slightly paler in colour than the male and the reticulations are more clearly defined. The brown fascia is of a duller and less reddish tone and its central portion is often indicated only in outline as is also the triangular patch of the apical and hind marginal regions. The cilia of the front wings are brown from the apex for about two-thirds of their distance, where they shade into orange towards the anal angle. Those of the hind wings are orange. The orange rings of the body are more distinct than in the male. The female, when at rest, might easily be passed over as a pale example of *T. heparana*.

"There is little variation in the fore wings, beyond one individual being slightly darker or lighter than another, but the black scaling of the hind wings is subject to very considerable modification, chiefly in the males. In the paler examples, the black border is represented by a slender line, just within the fringes, and any further black scaling is hardly discernible. In other specimens the border is black and black scales are scattered along the veins; while in the darkest individuals, the black scaling spreads over the wing so thickly as to almost obscure the orange colour."

Habits.

General observations on the life-history of the moth were begun in August, 1921, but no detailed studies were then made. Detailed observations were, however, commenced towards the end of May, 1922, pupae being collected from the glass-houses at Kew, the first moths emerging from these during the last week in May. The moths were then confined in cages containing young carnation plants. Food was provided in the form of sugar solution on a sponge and on blotting paper but the moths were never observed feeding. The duration of the life of the adult insect varies from 9 to 19 days, the sexes being approximately in equal proportion. Table I shows the proportion of the sexes to one another and the duration of adult life.

Table I.

Emergence 1922	Sex	Death 1922	Duration of adult life (days)
23. iii.	♀	3. iv.	12
6. v.	♀	19. v.	14
16. v.	♂	25. v.	10
24. v.	♀	9. vi.	16
25. v.	♂	29. v.	5*
,,	♀	5. vi.	11
,,	♂	3. vi.	10
26. v.	♀	5. vi.	10
27. v.	♂	9. vi.	14
,,	♂	,,	14
30. v.	♀	,,	10
21. vi.	♂	10. vii.	19

* Deformed specimen.

The moths are inactive during the day; it was usual for a moth to remain motionless in one spot all day long. In the greenhouses moths are rarely found during the day-time, unless disturbed from plants when they would fly a short distance and again settle down. In the early morning sun and towards dusk the adults become active and on summer evenings they were found in the open, in numbers flying about along with *T. unifasciana* in the neighbourhood of privet hedges where they had passed their larval and pupal stages. The male is much more active than the female—its flight being erratic and agitated; the female rarely flies, but when it does its flight is steady and it does not dart hither and thither as the male usually does. The moths do not exhibit any marked attraction to artificial light—but in the laboratory it was found that they showed a tendency to keep at the side of the cage nearest the window.

Pairing.

So far as has been observed the female pairs only once in her lifetime, and that very shortly after the moths have emerged. In one case a newly-emerged male was put into a cage containing several females. It flew excitedly round the cage and paired immediately with one of the females. In this instance pairing lasted 1½ hours.

Egg-Laying.

The female commences egg-laying almost immediately the act of pairing has been completed. In the laboratory eggs were laid occasionally during the day but much more frequently oviposition took place overnight or in the early morning and egg-masses were found in the morning which had not been present the previous evening. This confirms observations made in the open when the moths were found to be active in the early morning and in the evening. The eggs are laid in masses usually on the upper surface of the leaves of the host plant but occasionally masses are to be found on the under surface also. This position is, however, not common. In captivity in the laboratory, females have laid two or three batches of eggs on the under side of leaves of carnations and privet —in addition to several egg-masses on the upper surface.

THE EGG-MASS. (Fig. 1.)

The egg-mass consists of a number of eggs which overlap, resembling the shingles of a roof, and the shape of the mass, as a whole, apparently varies according to the shape of the leaf upon which it has been deposited. In the case of the long narrow leaf of the carnation the egg-mass is correspondingly long and narrow, while on the broad leaf surface of privet the mass assumes a distinct oval form. The eggs are laid in a number of masses which vary in size, the first laid masses being the larger, consisting in some cases of as many as 200 eggs, while the last deposited consist of only 10–20 eggs. The duration of egg-laying of a single female was found to be approximately 12–14 days during which 7 or 8 egg-masses were laid.

It is a common occurrence to find egg-masses deposited on the glass sides of breeding cages in the laboratory; on plant stakes and on the windows of green-houses. All fertile egg-masses laid on the leaves of host plants in the laboratory hatched, as also did a large proportion of those laid on glass, but, frequently, these latter did not mature.

The flat egg-mass is at first light green in colour and is surrounded by a mucilaginous substance. Each egg, oval in shape, consists of a large mass of yolk surrounded by an outer wall which presents a regular reticulate appearance. The "cells" of the reticulations were found, on microscopic examination, to be generally 5-sided. Towards the edges of the egg they appeared more or less elongated. No micropyle is visible.

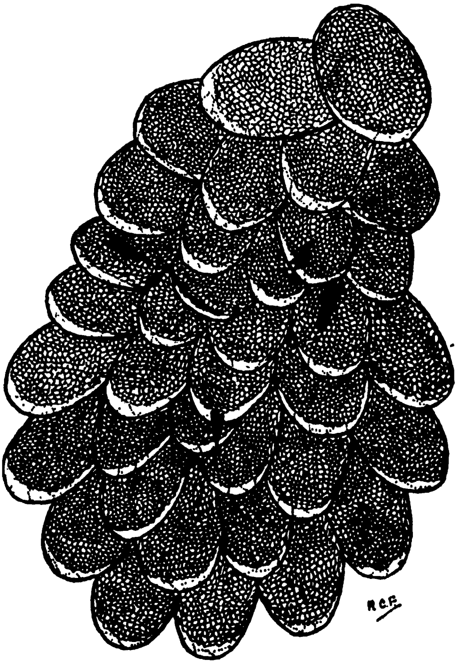


Fig. 1. Egg-mass of *T. pronubana* (magnified).

Incubation Period. (Table III, p. 402.)

The first egg-mass was laid in the laboratory on the leaves of carnations on May 27th, 1922, and egg-laying continued till the beginning of July. Conditions in the green-houses approximated to laboratory conditions but eggs had evidently been laid there prior to the end of May. When work was begun in May, 1922, larvae were present in the green-houses of a size which seemed to indicate that eggs had been laid in April. This was confirmed in spring, 1923, when it was observed that the first moths of the year commenced to emerge in the glass-houses from the end of March onwards.

The incubation period of the eggs varies from a minimum of 12 days to a maximum of 24 days, as far as observations have shown. The average period is 16–17 days. In 1922, eggs laid between 27th May and 10th June all hatched within a period of 12–13 days. The average temperature during this period was 69·6° F. On the other hand, eggs laid between 22nd June and 2nd July had an incubation period of 18–19 days. This longer period is explained by a fall in temperature. The average temperature during the period fell to 62·7° F. In May, 1923, the maximum incubation period obtained in the experiments (24 days) was accompanied by a still lower temperature—an average of 55·7° F. over the period. It is thus clearly shown that the incubation period of the eggs depends on temperature conditions. Moisture is, in all probability, another factor governing the development of the egg-masses. The death of many of the egg-masses laid on the glass sides of the breeding cages may be explained in this way.

Development.

The development of the eggs, as seen by the naked eye and low power hand lens, shows several successive changes in colour of the egg-mass. At first bright green, the mass becomes gradually darker green in colour and eventually each egg shows two small dark dots representing a pair of eyes. The egg-mass thus assumes a dark green colour—dotted all over with small dark brown points. The next stage of development is the appearance of the mandibles as dark objects and the egg-mass, as a whole, becomes greyish-green in colour. Just prior to hatching, the young larvae can be seen within the egg-shells actively opening and shutting their mandibles and twitching their abdomens. The larvae, on emerging, gnaw a hole in the egg-shells, push their heads through and thus rapidly escape. The first young larvae emerged in the laboratory on 7th June.

Table II.

The following table shows the number of egg-masses laid by two single females which had been fertilised and isolated.

Emerged	Pairing	Host	Oviposition and number of eggs per mass									Masses	Eggs
			1	2	3	4	5	6	7	8	9		
22nd June	22nd June	Carnation	22nd	23rd	25th	25th	26th	28th	5th	5th	6th	9	
			138	54	66	51	33	30	4	2	3		380–400
25th June	25th June	Privet	25th	26th	27th	28th	30th	2nd	2nd	3rd	6th	9–10	
			180–200	100	67	74	32	90	40	20	20		600–700

Table III.

Tabular Statement of Incubation Period of Eggs.

Host	Deposition	Hatching	Incubation period (days)
Carnation	28. v. 22	7–8. vi. 22	12
„	„	10. vi. 22	13
„	30. v. 22	11–12. vi. 22	12–13
On glass of cage	„	12. vi. 22	13
„ „	31. v. 22	11–12. vi. 22	12
Carnation	22. vi. 22	10. vii. 22	18–19
„	25. vi. 22	12. vii. 22	18
„	26. vi. 22	13. vii. 22	18
Privet	25. vi. 22	13. vii. 22	18
„	28. vi. 22	15–16. vii. 22	18–19
„	3. vii. 22	21. vii. 22	19–20
„	30. iv. 23	20. v. 23	20
Carnation	3. v. 23	26. v. 23	23–24
„	18. v. 23	7. vi. 23	21

Av. temp. 28th May–12th June, 1922 = 69·6° F.
„ 22nd June–7th July, 1922 = 62·7° F.
„ 30th April–7th June, 1923 = 55·7° F.

The Larva.

In the subsequent descriptions of the larval stages and duration of instars it is necessary to call attention to the fact that the observations were carried out during spring and summer, *i.e.* on the larvae of the summer brood. The duration of the instars, therefore, cannot be taken to refer to the larvae of the winter brood, where feeding and, consequently, growth is much slower and where the length of similar instars varies to even a greater extent than in larvae of the summer brood.

The arrangement of the setae or hairs which are found on the bodies of many lepidopterous larvae has been studied by Dyar (1901) and, within recent years, by Fracker (1915). In his *Classification of Lepidopterous Larvae*, the latter states that "the larvae of the family Tortricidae are of sufficient economic importance to warrant the expenditure of considerable time in the study of their structure." Accordingly, it was decided that it would be of importance to study the setal arrangement of the larva of *T. pronubana* on the lines of Fracker's work.

It was found that the 1st stage larva alone differs in its setal arrangement from the remaining stages—the newly-emerged larva lacking certain setae which appear at the first moult. Fig. 2 shows the distribution of the setae on the 1st stage larva while Fig. 7 shows the arrangement in the remaining stages (2–7).

Where differences occur in the external morphology of the larva at its various stages, these are noted; otherwise, the detailed description of the full-grown larva (p. 408) also applies to the younger stages.

1*st Stage Larva.*

Length 1¾–2 mm. A series of measurements of the head capsule varied from ·23–·26 mm. in breadth. The head is dark brown to black in colour. The thoracic shield is not prominent and is slightly darker than the rest of the body, being greyish-brown in colour. The remainder of the body is pale yellowish-green. The spiracles are elliptical with a dark margin. The prolegs are provided with uniordinal crotchets or hooks, *i.e.* all are more or less of one size. The crotchets number 9–10 per leg and are arranged in a circle which is complete on all legs with the exception of the anal proleg where the circle is not closed.

Arrangement of Setae. The larva is well clothed with stiff erect whitish bristles or setae and their distribution on the thoracic and abdominal segments is diagrammatically shown in the setal maps (Figs. 2 and 7). The maps have been prepared according to Fracker's method of representing the arrangement of setae. Each segment of the larva is shown on the map by a rectangle, the upper side of which corresponds to the mid-dorsal line and the lower side to the mid-ventral line.

The distribution of the setae of the 1st stage larva of *T. pronubana*—which, it will be recalled, measures only 2 mm. in length—cannot be observed without the aid of a binocular or a compound microscope. In studying this stage, in order to obtain a clear idea of the number and arrangement of the setae it was found necessary to use a compound microscope (Obj. ⅔″) examining the larva, illuminated by artificial light and using a condenser. Studies of mounted specimens were also made.

The setae arise from slightly raised tubercles and in some cases several setae arise from the one tubercle—for example, the two setae immediately beneath the spiracle on the first eight abdominal segments both arise from the one tubercle. The tubercles are paler in colour than the surrounding epidermis, and, on microscopic examination, it is seen that they are devoid of the minute spines which are found all over the rest of the larval skin (Fig. 8). The setae are simple and arise from pits on the tubercles. They vary in length in different parts of the larva, the head carrying setae of the greatest difference in length.

Setae which are present in the 1st stage larva are termed by Fracker "primary": while those appearing at moult 1 are called "sub-primary." A comparison of the two setal maps (Figs. 2 and 7) shows that on the prothoracic segment, the only difference between the first and subsequent stages is the absence in the former of one seta of the group A, which is found slightly below and anterior to the prothoracic spiracle. The same difference occurs on the remaining thoracic segments (cf. A 2, A 3—Fig. 7).

The setae on the abdominal segments are numbered after Dyar (1901). Seta V (Fig. 7) is not found on any segment in the 1st stage larva; it arises at moult 1 and is thus sub-primary. On segment 9, groups IV and VI have each one seta less in the 1st stage larva. The arrangement on segment 10 (Fig. 7) is similar in all stages.

Habits and Duration of Instar. On emerging, the young larvae are extremely active and immediately wander rapidly over the food plant. They are markedly positively phototrophic, always crawling towards the light. Never at any other stage in its development was the caterpillar so active—crawling rapidly about and, if in the least disturbed, dropping at once suspended by a long silken thread. In the laboratory the larvae were found to congregate at the tips of the leaves of the carnation plants, hanging in festoons on their threads and spinning quickly up and down. Adkin (1908) states that the newly-emerged larvae wander long distances from their birthplace before settling down to feed. Observations at Kew confirmed this, to a certain extent, but there was little difficulty in getting the young larvae to settle on the plants on which they were born. In the green-houses, on the other hand, it was quite evident that larvae wandered from plant to plant and were thus rapidly scattered, although all derived from one particular egg-mass. This wandering habit of the 1st stage larva undoubtedly, as Adkin has pointed out, gives the young caterpillar every opportunity of obtaining a plentiful food supply.

At this stage the larvae settle on the upper surface (occasionally on the lower surface) of the tips of leaves, in the leaf-axils and in the flowers of carnations—either singly or two or three together. After settling, a shelter of silk is soon spun and the caterpillar commences feeding upon the upper epidermis of the leaf. On other hosts—for example on those the leaves of which have a broad surface—it was a common occurrence to find two leaves spun together, the upper surface of one to the lower surface of the other and, between the leaf surfaces, two or three larvae feeding on the epidermis. Where larvae settle in flowers, *e.g.* carnation, the petals are spun together and perforated with several holes produced by the feeding of the caterpillars. Occasionally it was found that the 1st stage larva fed for a short time in one place and then wandered on to another. Usually, however, it remained in one spot till after the first moult.

All the larvae which emerged on 8th June and had been feeding in the laboratory on carnation plants moulted for the first time on 18th June. The duration of the 1st instar was therefore 10–11 days. This period was again observed in subsequent batches of larvae which were reared in the laboratory.

At moult 1—as also at all other moults with the exception of the last—the cast skin is eaten by the newly-moulted larva and only the cast head capsule is left to indicate the occurrence of a moult. This habit has been shown to be also true of the Holly Tortrix Moth (*Eudemis naevana*, Hb.) (Huie, 1917).

2nd Stage Larva.

Length 3–4 mm. Breadth of head ·30–·33 mm. This stage can readily be distinguished from the 1st stage larva by the colour of the head—which is distinctly light brown—as compared with the dark head of the previous stage. The thoracic shield is more distinct and there are now indications of dark markings appearing upon it. The colour of the body is of little value in distinguishing the stage. In general, the larva is rather more greenish in colour, as a result of feeding, but it was found that, as in all other stages, the colour varied with the food plant. In some cases the larva maintains the pale yellow-green colour of the first stage but in the laboratory, where larvae were reared on carnations, they showed a more pronounced green in their coloration. The crotchets of the prolegs are uniordinal—12–15 crotchets per leg.

Habits and Duration of Instar. The habits and method of feeding of this stage are similar to those of the 1st stage larva. After the first moult the caterpillars continue feeding in the same position as before, spinning a slightly longer and denser covering and, as a result of their increased size, eating more rapidly, but still feeding upon the epidermis of leaves or upon the petals of the flowers.

The duration of the 2nd instar was again found to be, on the whole, constant for larvae of the same brood. Larvae which moulted for the first time on 18th June moulted again on 27th–28th June. The duration of the 2nd instar is thus 10–11 days—the same as that of the 1st instar.

3rd Stage Larva.

Length 6–7 mm. Breadth of head ·42–·48 mm. The head is light brown in colour with varying darker markings, now more distinct. The thoracic shield is also light brown and has four dark markings towards its posterior margin. The hooks on the prolegs are uniordinal, 15–20 (approx.) per leg.

Habits and Duration of Instar. At the commencement of this stage the larvae continue feeding as before but towards the end of the instar and throughout the remaining instars, not only the epidermis, but the entire leaf is eaten through. As the larvae grow older and larger they feed under a more dense silken covering and are usually completely hidden from sight by the rolling of the leaf or leaves on which they are feeding. The larvae do not remain on one leaf throughout their life but, as food becomes scarce and exhausted, through the withering of attacked leaves, they move to fresh food and again spin themselves a new home and re-commence feeding. The 3rd stage larva feeds singly; it was unusual to find more than one on a leaf. As the larvae grow older they become less active. Their readiness to hang down on threads when disturbed, so noticeable in the first stage, is not nearly so marked in the remaining instars.

The duration of the 3rd and subsequent instars varies very considerably. Owing to the habit of the larvae of feeding completely hidden between spun together leaves and, further, as a result of their wandering about on the plant, it was found extremely difficult to follow the development of individuals. The duration periods, therefore, of this and the remaining instars, are probably only approximate. Larvae of one brood (*i.e.* emerging from the same batch of eggs) were, however, followed throughout their development and the results obtained give approximately the length of duration of the different stages of larval growth. In rearing a batch of larvae emerging at one time, as was done with *T. pronubana*, it was of interest to notice that during the first two larval stages the duration of the instars remained approximately the same for individual larvae. In the third and subsequent stages, however, some larvae grew much more slowly than others and, as a result, the duration of the instars varied to a great extent.

The duration of the 3rd instar varied from 9–20 days. The first 3rd stage larva moulted on July 6th—*i.e.* moult 3.

4th Stage Larva.

Length 8–10 mm. Breadth of head ·62–·67 mm. The head is light yellow-brown with various darker markings.

The thoracic shield is light brown with four dark patches on its posterior border, as in the 3rd stage larva.

In the laboratory, the larvae reared on carnations had now begun to assume a more pronounced olive-green colour. The tubercles from which the setae arise are more prominent.

The crotchets of the prolegs are still uniordinal and number approximately 25–30 per leg.

Duration of Instar. As in the 3rd instar, there is a great variation in the duration of the 4th instar. The first 4th stage larva moulted (moult 4) on 18th July, and cast head capsules of 4th stage larvae were found continuously in the laboratory experiments up till the middle of August. The minimum length of the instar was 8 days and the maximum observed was 20 days.

5th Stage Larva.

Length 10–12 mm. Breadth of head ·78–·85 mm. The markings on head and thoracic shield are similar to those found in the 4th stage larva.

The body is more distinctly olive-green in colour with tubercles rather more prominent than heretofore.

The prolegs now are provided with two types of crotchets—some hooks being longer and others shorter. As yet, no definite arrangement of the two types is found. The 5th larval stage therefore differs from the preceding four instars, in the structure of the prolegs. The crotchets again number approximately 25–30 per leg.

Duration of Instar. The duration of this instar again varies. Two larvae were found on 21st July which had apparently moulted for the fifth time. Their cast head capsules were not, however, found. This phenomenon was noticed on several occasions—6th stage—or apparently 6th stage larvae, being found but no cast heads were obtained to confirm the occurrence of the suspected moult.

In the laboratory moult 5 was recorded from 27th July and at intervals onwards from that date.

The minimum duration of the 5th instar was 9 days—the maximum was not determined.

6th Stage Larva.

Length 12–15 mm. Breadth of head ·93–1·1 mm. Head light yellow-brown with numerous dark patches as found in the 4th and 5th stage larvae. Thoracic shield yellow-green with sundry markings at its posterior margin.

The dorsal surface of the body is of a darker olive-green than the ventral surface—the anal segment on the dorsal surface is, however, also light green in colour. The tubercles are paler than the rest of the body and the setae long and distinct.

The crotchets of the prolegs are now distinctly biordinal and are arranged so that long and short hooks alternate (Fig. 8). The crotchets number approximately 40 per leg.

Duration of Instar. The first 6th stage larva moulted on 3rd August. The duration of the instar again varied but observations showed a minimum of 10–11 days. In two recorded instances the duration was 17–18 days, but it was not possible to determine the maximum period.

7th Stage Larva. The full-grown Larva.

General Description. The full-grown larva (Fig. 3) measures from

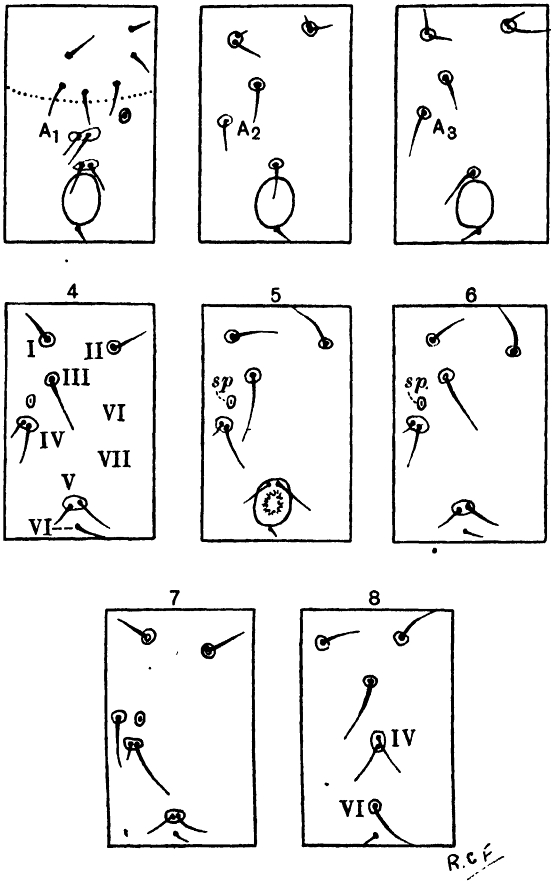


Fig. 2. Setal maps of 1st stage larva.

Map 1, Prothorax; 2, Mesothorax; 3, Metathorax; 4, Abdominal segments 1 and 2; 5, Abdominal segments 3, 4, 5, 6; 6, Abdominal segment 7; 7, Abdominal segment 8; 8, Abdominal segment 9. *Sp.* =Spiracle.

I, II, III, etc. =Names of groups of setae after Dyar (1901). A 1, A 2, A 3—compare with A 1, A 2, A 3, Fig. 7.

15–20 mm. in length and is cylindrical in shape, tapering slightly towards both ends. The head, which varies from 1·2–1·5 mm. in width, is of a glabrous yellow-brown colour with various dark markings, the size and position of which differ in individuals.

The thoracic shield is green—rather paler than the dorsal surface of the segments of the body—and is irregularly marked with dark brown patches, most typically four more or less triangular markings, along the posterior margin of the shield.

The dorsal surface of the mesothorax and metathorax and of abdominal segments 1–9 is olive-green in colour, while the ventral surface and the entire 10th segment are of a much paler green.

The spiracles are broadly elliptical in shape and have dark margins: the prothoracic and 8th abdominal spiracles are larger than the others and the 8th is slightly more dorsal in position. With the exception of the prothoracic spiracles, which are situated in the posterior half of the prothorax, all the spiracles are found towards the anterior boundary of their segments and are placed between two tubercles which bear setae. A single seta arises just dorsal to the spiracle and two, with their origin on one tubercle, just ventral and slightly anterior to it.

There are numerous folds in the body wall of the larva (Fig. 3). Each segment is divided dorsally by a transverse fold towards the centre of the segment, while a longitudinal fold runs just above the legs, throughout the entire length of the larva, with the exception of the prothoracic and 10th abdominal segments.

The larva is well clothed with stiff white hairs, the arrangement and distribution of which are discussed below (p. 415).

Detailed Description.

The Head (Figs. 4 and 5). The head is slightly smaller and narrower than the thoracic shield and can thus be partly withdrawn beneath the shield. In a normal position the latter extends slightly over the occipital region of the head. It is not depressed and is glabrous with, as previously stated, sundry dark markings at its base.

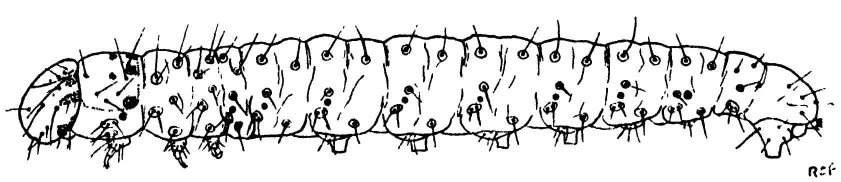


Fig. 3. Full-grown larva of *T. pronubana*. × 5 (approx.).

The ocelli, which are situated on a dark patch, are 12 in number—six on each side of the head—immediately posterior to the antenna. The first five ocelli are arranged in a curve and are just visible dorsally while the sixth is more ventral in position.

The dorsal aspect of the head, including the arrangement of the hairs, is represented in Fig. 4.

The Antennae are 3-jointed. The basal membrane of soft chitin is well marked and gives the antenna the appearance of having four joints. The basal joint can be withdrawn within the basal membrane. The middle joint is larger than the other two and is dark brown in colour. It carries two bristles, one, the longer, arising ventrally, and the other, the shorter, which arises from the dorsal surface. At the anterior end of this joint there are two small cones. The terminal joint is small and also carries at its apex a fine bristle and several small cones, one of which terminates in a fine hair.

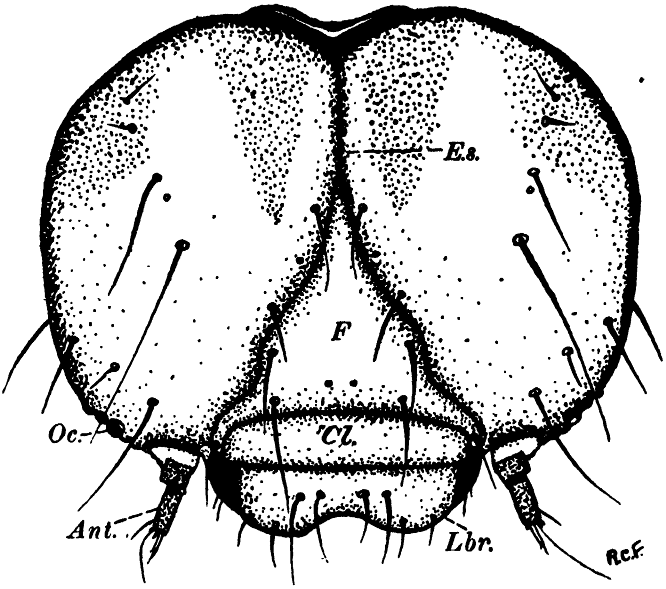


Fig. 4. Dorsal aspect of head of full-grown larva (magnified), showing distribution of hairs. *E.s.* = Epicranial suture; *F.* = Frons; *Cl.* = Clypeus; *Oc.* = Ocellus; *Lbr.* = Labrum; *Ant.* = Antenna.

The Labrum is twice as long as it is broad. The anterior margin, which projects inwards in the middle line, and divides the labrum anteriorly into two lobes, is more strongly chitinised than the remainder of the labrum. The "plate" of chitin thus formed is visible from the ventral surface. Dorsally, on each side of the middle line, the labrum carries six bristles and their arrangement agrees with that figured by Trägårdh (1917) for the labrum of *Laspeyresia strobilella*. Bristles III and VI are marginal in position; IV and V are submarginal, while I and II are situated close to the middle line, some distance from the margin of the labrum and in line with bristle VI.

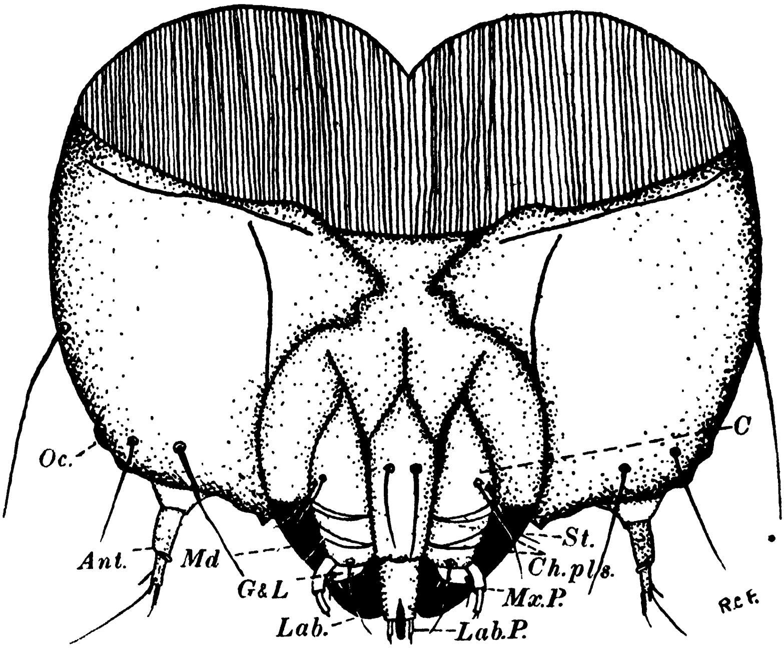


Fig. 5. Head of full-grown larva. Ventral aspect (magnified), showing mouth-parts and hairs.

C. = Cardo; *St.* = Stipes; *Ch.pls.* = Chitinous plates; *Ant.* = Antenna; *G.* & *L.* = Galea and Lacinia; *Mx.p.* = Maxillary palp; *Lab.* = Labium; *Lab.p.* = Labial palp; *Md.* = Mandible; *Oc.* = Ocellus.

Ventrally the labrum carries no bristles but is provided with six thorn-like chitinous projections, three on each lobe, towards its anterior margin. II is distinctly smaller than I and III, and in this apparently differs from Trägårdh's figure of the *Laspeyresia strobilella*. Further, the two chitinous plates which are also present towards the centre of the labrum of the above species, one on each side of the middle line, are in the labrum of *T. pronubana* entirely absent. The ventral aspect of the head and the mouth parts is shown in Fig. 5.

Mouth Parts (Fig. 5). In the ventral view the mandibles are seen lying behind the maxillae and the labium. The maxillae lie one on each side of the labium and each is connected to it by a strong chitinous rod. Each maxilla consists of the cardo and stipes, the latter being provided with two bow-shaped chitinous plates having the appearance of joints, but which, according to Börner (1907), are merely strengthening plates of chitin. Each plate bears a bristle at its anterior border, while some distance behind the posterior plate and on the border of the cardo and stipes, two other bristles arise of slightly differing length.

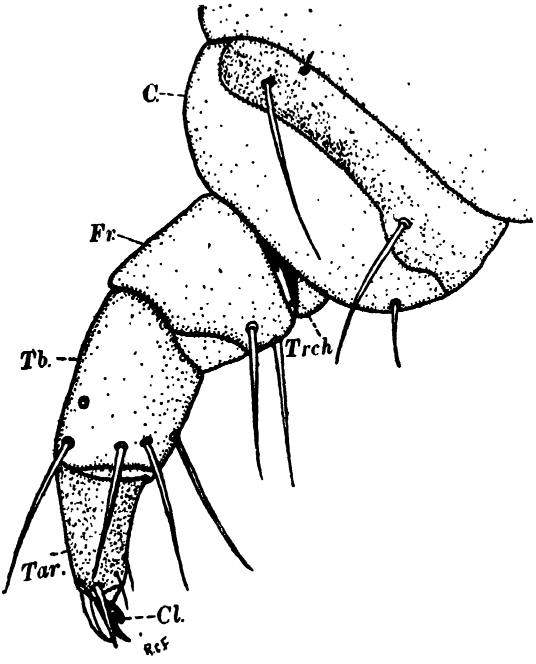


Fig. 6. Thoracic leg of full-grown larva. Posterior surface (magnified).
C. = Coxa; *Trch.* = Trochanter; *Fr.* = Femur; *Tb.* = Tibia; *Tar.* = Tarsus; *Cl.* = Claw.

The *Stipes* carries a two-jointed maxillary palp—the terminal joint of which bears several minute papillae and hairs. Also arising from the stipes and situated between the maxillary palp and the labium lie the fused galea and lacinia (or what apparently corresponds to the galea and lacinia). This structure carries six chitinous projections of varying size and shape. They are as follows: two small cone-like projections, each with two joints, the second of which is the smaller; two flat plate-like bristles, differing from ordinary setae in being broader and flatter and not tapering gradually to a fine thread-like point; a minute bristle-bearing papilla and an ordinary seta arising close to the maxillary palp.

The *Labium* lies between the two maxillae and is modified, as in other Lepidopterous larvae, to form the spinneret, at the tip of which the silk glands terminate. The labium is separated on each side from the maxillae by a stout rod of chitin. At the anterior end, on each side of the spinneret, is a small labial palp, two-jointed and terminating in a fine hair. The

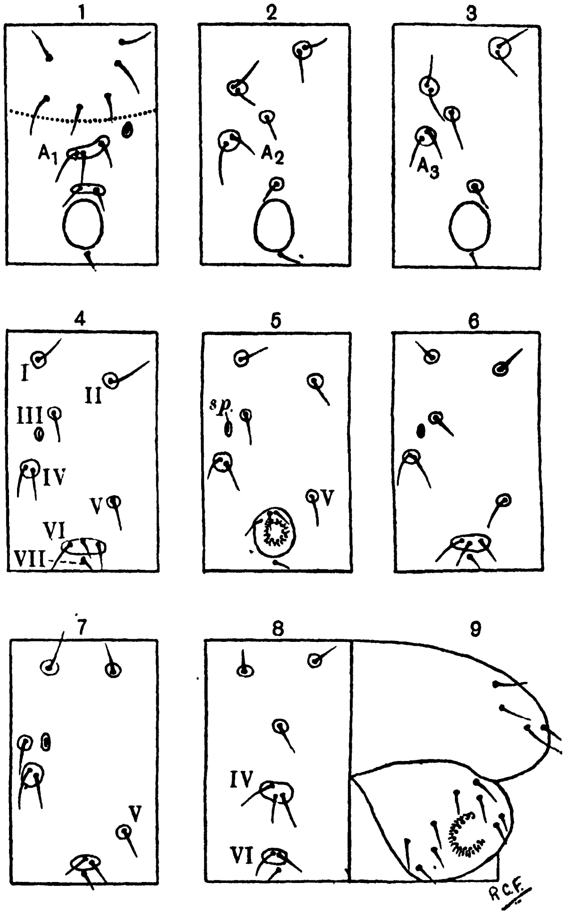


Fig. 7. Setal maps of larval stages, 2 and 7.

Map 1, Prothorax; 2, Mesothorax; 3, Metathorax; 4, Abdominal segments 1 and 2; 5, Abdominal segments 3, 4, 5, 6; 6, Abdominal segment 7; 7, Abdominal segment 8; 8, Abdominal segment 9; 9, Abdominal segment 10. *Sp.* = Spiracle.

I, II, III, etc. = Names of setae after Dyar (1901). *Note.* Presence of seta V—absent in 1st stage larva.

labium carries two setae which arise slightly behind the posterior pair of bristles on the stipes.

Both in the dorsal and in the ventral aspects of the head, the mandibular condyle is visible as a small knob-like projection, situated between the mandibles and the antennae, but close to the former.

Thoracic Legs (Fig. 6). The thoracic legs are 5-jointed and consist of coxa, trochanter, femur, tibia and tarsus. Viewed from behind, the arrangement of setae and chitinous plates is as follows:

The basal third of the *coxa* consists of a chitinous plate at the posterior margin of which a minute bristle is situated. Two long setae arise from the middle of the plate one on either side. A fourth seta is situated immediately on the inner margin of the coxa.

The *trochanter* is a small joint and is provided with a chitinous plate and a clear area of articulatory chitin at its anterior margin. At each end of this clear area is a minute bristle.

The *femur* carries two bristles: one arises towards the inner margin and the other has its origin almost on the anterior surface of the joint.

The *tibia*, which is separated from the femur and tarsus by a clear area of soft chitin, bears four bristles or setae arising towards its anterior margin. A pit, indicating the possible origin of a fifth bristle, is seen towards the outer margin of the joint.

The *tarsus* is darker in colour than the other joints and terminates in a claw which consists of a sharp curved hook and a smaller blunt pad-shaped piece of chitin at the base of the larger claw and on its inner surface. Two short bristles arise from the inner margin, on the anterior third of the tarsus. In addition, the tarsus bears at its anterior end two thorn-like bristles, more plate-like than those arising from other parts of the leg. One of these bristles arises from the outer margin of the joint and the other over-lies the claw, with its insertion slightly posterior to the base of the claw.

On the anterior surface of the leg the joints are devoid of bristles, with the exception of the tibia which carries two setae towards its anterior margin and situated one on either side of the middle line of the joint.

Prolegs (Fig. 8). The prolegs of abdominal segments 3–6 are crowned with a complete circle of biordinal (alternating, longer and shorter) crotchets which number between 50 and 60 per leg. The anal prolegs do not have a complete circle—the crotchets extending only rather more than half-way round the foot. The crotchets are thickened plates of chitin which are bent at the apex in the form of a hook. For some distance around the circlet of hooks, but on the leg itself, are numerous minute hair-bearing papillae.

A tubercle, bearing three setae, and which is free from any spines, is situated just above each proleg. A single seta, arising from a tubercle, which is also free from any spines, is seen beneath each proleg and close to the mid-ventral line. The base of the proleg is devoid of the minute spines which cover the rest of the epidermis of the body of the larva.

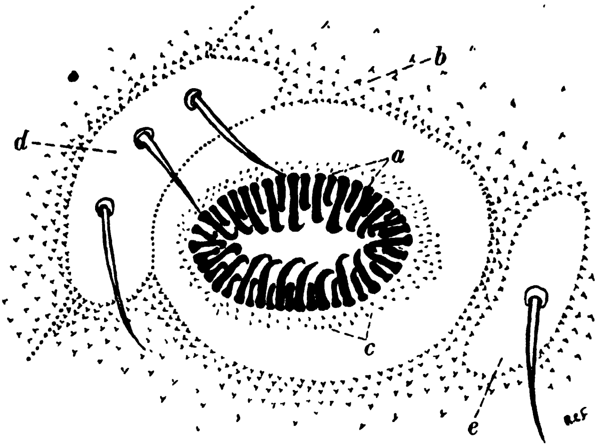


Fig. 8. Proleg (ventral view) of full-grown larva (highly magnified).

a, Biordinal crotchets; *b*, Small spines on skin of larva but which are absent on part of leg and on each tubercle from which setae arise; *c*, Hair-bearing papillae around base of crotchets; *d*, Group of 3 setae—Group VI; *e*, Single seta—Seta VII.

Arrangement of Setae. Attention has already been drawn (p. 403) to the setal maps (Figs. 2 and 7) which have been prepared to illustrate diagrammatically the number and distribution of the setae in the 1st stage and subsequent stage larvae.

Adkin (1907–8) figures the dorsal view of a segment of the larva of *T. pronubana* showing the arrangement of the hairs. It is apparent, however, that his figure can represent the dorsal view of the first eight abdominal segments alone.

In his classification of Lepidopterous larvae, Fracker (1915) figures a segment of the larva of *Cydia pomonella* as representing the family Tortricidae. While the larva of *T. pronubana* agrees in most points with Fracker's conception of the arrangement in the Tortricidae, certain differences and variations occur which it may be of interest to note. For example, the arrangement on the 9th abdominal segment differs considerably from that on the corresponding segment of the larva of

C. pomonella. Further, in one case a variation was found in the number of setae composing Group VI (Fig. 7) on segment 8 of the abdomen. According to Fracker, the genus Tortrix, in common with several other stated genera, is characterised by this group being disetose. In one specimen of *T. pronubana* it was found that this group consisted of two setae on one side of the segment and of three setae on the other side. This non-symmetry was, however, only once observed. In all other respects the larva of *T. pronubana* was found to agree with the characters given by Fracker for the genus Tortrix. On the 8th abdominal segment, for example, the seta which, on the first seven abdominal segments arises dorsally and slightly posterior to the spiracle, on this segment invariably is anterior to and on the same level as the spiracle. Fracker believes that this is general in the Tortricidae except for a few rare cases.

Infra-Anal Comb. Situated beneath the anus, which is almost dorsal in its position at the apex of the 10th abdominal segment and posterior to the anal prolegs, on the ventral surface of the last segment there is a chitinous flap which is prolonged into a number of sharp bristles. This flap or comb (Fig. 9) is present in all larval stages. The number of bristles varies from six to ten—the commonest number, however, being eight. In some cases the bristles are pointed but others are bifid or have a jagged end as though they had been broken. Examination of a number of combs never once showed one with a series of pointed bristles. It may

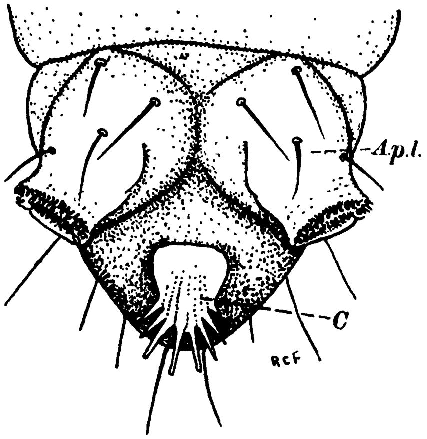


Fig. 9. 10th abdominal segment. Ventral view. Showing anal prolegs (magnified) and infra-anal comb.

A.p.l. = Anal proleg; *C.* = Infra-anal comb. (*Note*, 8-pointed.)

thus be assumed that the points have been broken either accidentally in making the microscopic preparations required for examination, or have been worn down through use. The bristles are arranged in a fan-like fashion according to their size. Where ten bristles are present the additional two are minute. In a dorsal view of the last segment of the larva, the apices of the bristles of the comb can be seen projecting beyond the posterior border of the segment.

The flap is hinged to the ventral surface of the body-wall and is capable of moving backwards and forwards through an angle of 180°. The apices of the flap can be brought together and closed in a fan-like manner. This flap appears to correspond with the "infra-anal lobe" described by Packard (1898) who states that his attention was first called to this lobe when examining some Geometrid larvae, where it takes the form of a thick conical fleshy lobe, ending in a hard chitinous point. He suggests that its function in the Geometridae is to aid in tossing the pellets of excrement away, so as to prevent contact with the body. It would appear that the hinged flap with its numerous hard chitinous bristles which is found in the larva of *T. pronubana* has a similar function. It is worthy of note that although the larva feeds beneath a silken web knitting closely together leaves or petals, its feeding ground was never found full of excrement; yet all around the twigs of privet and carnation plants, on which the larvae were feeding, in the laboratory, the excrement was plentiful. It is thus obvious that the pellets of excrement must have been ejected with some force from the home of the larva and tossed some distance away.

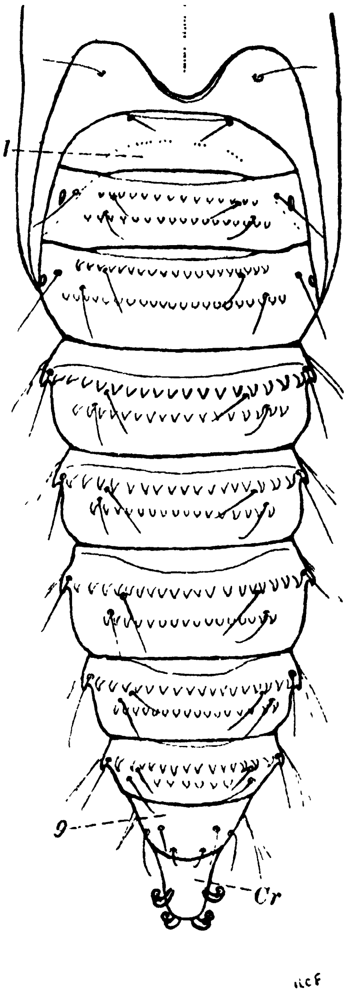


Fig. 10. Pupa of *T. pronubana*. Dorsal aspect of abdominal segments. × 12½. Showing arrangement of spines and hairs.

An anal-comb with eight points is found in the larva of *T. viridana* (Sich, 1915–6). It has, however, not been possible to ascertain whether such a comb is present in all members of the genus Tortrix or whether, indeed, it is to be found throughout the entire family Tortricidae. No mention is made of its presence in the larva of the Holly Tortrix Moth, *Eudemis naevana.*

COLOUR VARIATION IN LARVAE.

Reference has already been made to the variation in colour of the larvae which renders that character useless as a means of distinguishing the different larval stages. Observations made in the green-houses where larvae were feeding on a great variety of plants (see Appendix) showed that similar stages on the same plant varied to some extent in colour. Where a larva had been feeding on a yellow flower, *e.g. Cytisus fragrans*, its colour was very pale green with a more or less marked yellowish tinge. Such larvae transferred to the leaves of Cytisus gradually lost their yellowish tinge and became bright olive-green. Larvae on one plant in particular—*Nandina domestica*—were, with the exception of the 1st stage, always distinctly yellow in colour with a very slight trace of green. The larvae were feeding on the leaves of this plant in numbers: the plant was not in flower. Full-grown larvae reared on this host retained the yellow colour. A yellow dye is obtained from the wood of this and other plants belonging to the natural order Berberideae, an interesting fact which may be correlated with the yellow colour imparted to larvae feeding on its leaves.

Larvae reared on carnations in the laboratory were more or less of the same colour at corresponding stages but even in their case, also, there were differences in the shades.

It can be concluded that, as Poulton (1892, 1903) has shown from other Lepidoptera, the food plant has a distinct effect on the colour of the larvae feeding on it. At the same time, it was found that this effect was not the same on all individuals—larvae on the same hosts and at similar stages of growth, differing in colour from one another.

It was not, however, found that variations in colour of larvae resulted in marked variations of the adult moths. Pupae collected from many species of plants yielded adults differing slightly in density of coloration. In some specimens the markings on the fore wings were darker than in others. Adkin (1907) has drawn attention to considerable modification of the black scaling on the hind wings particularly in the male. It is probable that this slight variation in the adult may be correlated with the colour variation in the larva but time has not permitted of a study of this matter.

Duration of 7th Instar. The duration of the instar, so far as has been observed, varies from a minimum of 14 days to a maximum which was not determined. The first pupa was obtained in the laboratory on 14th August. From that date onwards pupae were obtained from larvae which emerged on 8th June. In two or three cases pupation did not take place till spring, 1923.

THE PUPA.

Pupation.

Just prior to pupation the fully-grown larva completely covers itself with a sheath of silk, rather denser than that spun in the previous stages of its development. The leaves are thus tightly woven together and the pupa is invisible unless its shelter is torn apart and the mass of silk threads broken. Pupation takes place in a rolled leaf, in the axil of a leaf or between two or more leaves spun together.

Description and Chaetotaxy.

The pupa is at first brown in colour but later darkens, in most cases becoming quite black. In a few cases, however, the brown colour persists throughout the pupal period. The size varies from 9–12 mm., the average being 10 mm. As a general rule, the males are rather smaller than the females but this is not absolutely constant.

Dorsal Aspect (Fig. 10). The head, prothorax, mesothorax, metathorax, wings and nine abdominal segments and the cremaster are visible in a dorsal view of the pupa. The 10th abdominal segment is not visible. The 2nd and 3rd abdominal spiracles are visible and their position as well as the arrangement of the spines and hairs on the abdominal segments are shown in Fig. 10. The 1st abdominal segment does not bear any spines. Segments 2–8 inclusive, bear two rows of backwardly directed spines, one row near the anterior border of each segment and the other towards the centre. Segment 9 typically is without spines but in one case a pupa was found with the 9th abdominal segment bearing a central row of three or four spines (Fig. 11). The spines on all segments are extremely fine-pointed and, viewed in a certain light, appear as though they terminated in fine hairs.

The cremaster (Fig. 11) carries four pairs of hooks, two pairs lateral in position, which arise from the dorsal surface of the cremaster, and two pairs arising terminally from its ventral surface.

The dorsal surface of the pupa bears several yellowish hairs and the

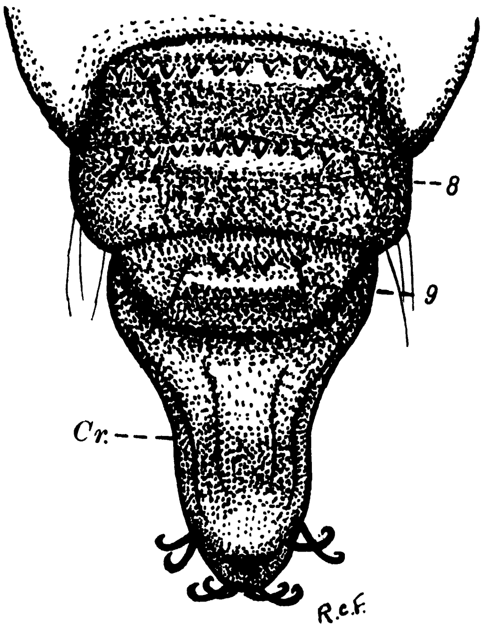


Fig. 11. Pupa of *T. pronubana*. Dorsal aspect of 8th and 9th abdominal segments and cremaster (highly magnified).

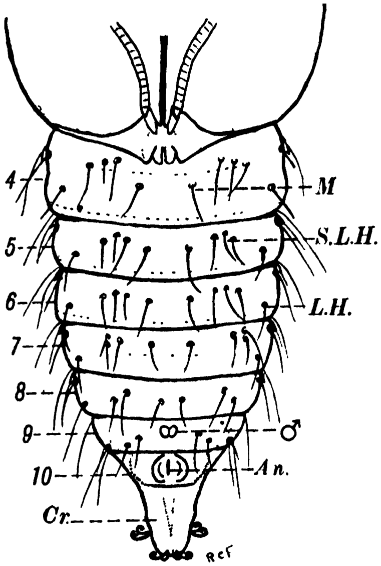


Fig. 12. Pupa. Ventral aspect of abdominal segments. × 12½.
4–10 = abdom. segments; *Cr.* = Cremaster; *An.* = Anus; ♂ = male sex character; *M.* Median Hairs; *S.L.H.* = Sub-lateral Hairs; *L.H.* = Lateral Hairs.

arrangement and distribution of these on the abdominal segments are also shown in Fig. 10. The hairs arise from pits and are not borne on tubercles as was found in the larva.

Ventral Aspect (Fig. 12). Abdominal segments 4–10 are visible from the ventral surface. The 10th segment carries the anus and is separated from the cremaster by an indistinct intersegmental line. The two pairs of terminal hooks on the cremaster arise from the ventral surface.

Numerous yellowish hairs are again found. Their arrangement is similar on segments 4–7, each segment bearing two single median hairs; two groups of three sub-lateral hairs, and two lateral hairs. The sub-lateral groups on segment 8 consist of two hairs only; while segment 9, although otherwise resembling the 8th segment, appears to lack the pair of median hairs. Segment 10 is without hairs and is indistinctly separated from the cremaster.

Lateral Aspect (Fig. 13). The first nine abdominal segments are easily distinguishable in a lateral view of the pupa. In the male chrysalis the 10th segment is not (in this view) visibly separated from the cremaster by one intersegmental line: in the female only an indistinct division is apparent. Thus, in neither sex, is the posterior boundary of the 10th segment well defined.

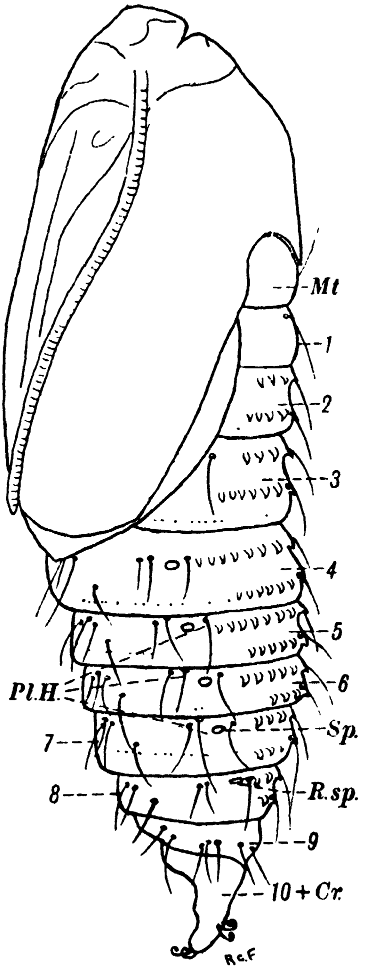


Fig. 13. Pupa. Lateral aspect. × 12½. *Mt.* = Metathorax; 1–10 = abdominal segments; *Pl.H.* = Pleural Hairs; *Sp.* = Spiracle; *R.sp.* = Rudimentary spiracle; *Cr.* = Cremaster.

Spiracles are visible on segments 4–7. The spiracles on the first three abdominal segments are hidden beneath the pupal wings. A rudimentary spiracle is present on the 8th segment. The arrangement of the pleural hairs is best described by reference to Fig. 13. In the lateral view of the pupa several of the dorsal and ventral hairs are also visible. The 10th segment and the cremaster do not carry any hairs.

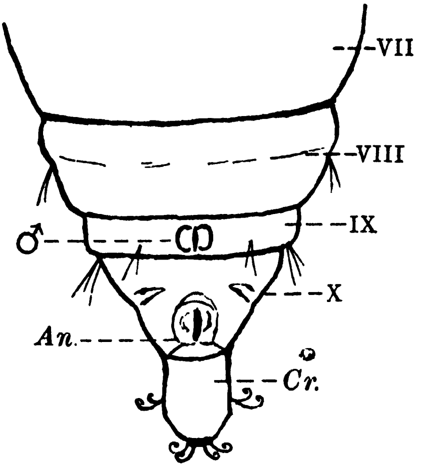


Fig. 14. Pupal sex character. Ventral surface of abdominal segments VII–X of male pupa of *T. pronubana.*

♂ = sex character; *An.* = Anus; *Cr.* = Cremaster.

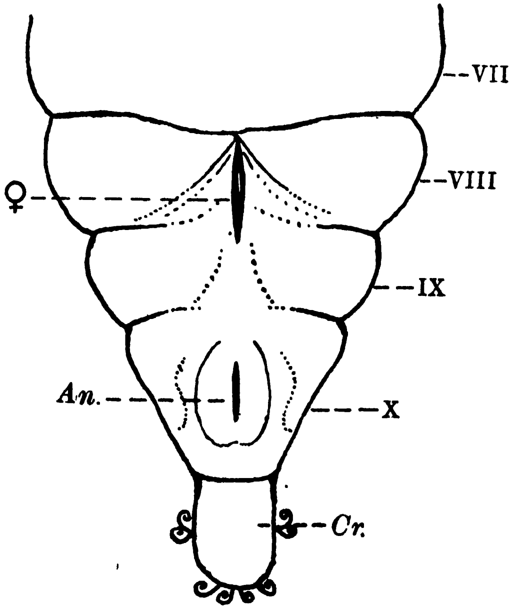


Fig. 15. Pupal sex character. Ventral surface of abdominal segments VII–X of female pupa of *T. pronubana.*

♀ = sex character; *An.* = Anus; *Cr.* = Cremaster.

Pupal Sex Characters.

A knowledge of sexual differentiation in the pupae of *T. pronubana* has proved invaluable during biological work. The external generative organs of Lepidopterous pupae have been described by Hatchett-Jackson (1890) and Poulton (1890). According to both authorities the male sex of Heterocera is distinguished by a well-defined linear depression in the middle line of the ventral surface of the 9th abdominal segment. This depression is guarded by two trianguloid tubercles placed one on each side of the middle ventral line. According to Poulton the furrow between the tubercles is the ancient opening of the pupal vas deferens and now corresponds externally to the termination of the ducts internally.

Hatchett-Jackson summarises the characters of all the female Heterocerous pupae he has examined by "a triangular forward extension of the sternal region of the 9th abdominal segment, invading the sternal region of the 8th together with either (*a*) a linear depression on the 8th sternal region and another at the apex of the triangular extension, *i.e.* actually on the 9th segment, or (*b*) a single depression close to, or in the apex of, the triangular extension and produced by a confluence of the two depressions before mentioned." He further adds that, as a result of this forward extension of the 9th abdominal segment, the intersegmental line between segments 9 and 10 is not continuous from side to side across the ventral surface. Poulton's observations confirm those of Hatchett-Jackson and, at the same time, he discusses the actual segmental relationship of the two depressions referred to in (*a*) above. The anterior opening, according to Poulton, indicates the opening of the bursa copulatrix and is always associated with the 8th abdominal sternite. The posterior opening, on the other hand, which is for the oviducts, varies in its position and is often obscure and unrecognisable and is frequently fused with the anterior aperture. Poulton states that it may occur immediately anterior to the apex of the triangular projection of the 10th segment, *i.e.* on the 9th sternite, or on the projection itself and so on the 10th sternite.

Examination of pupae of *T. pronubana* has shown that as regards pupal sex characters the species agrees in general with those given by Poulton and Hatchett-Jackson and which are summarised above. The following notes on *T. pronubana* in particular may be of interest:

Male (Fig. 14). On the 9th abdominal sternite there are two knob-like tubercles lying between which, in the middle line, is a linear furrow or depression. The tubercles are easily visible by means of a hand lens.

The intersegmental lines between segments 9 and 10 are continuous from side to side.

Female (Fig. 15). The 9th segment extends forward in the form of a triangular projection invading the 8th segment. The apex of the extension reaches, in the middle line, to the posterior boundary of segment 7. In the middle line and on the extension of the 9th segment there is a slit-like linear depression extending the whole length of the extension and provided on both sides with prominent lips. Thus two separate generative openings are not visible in the pupa of *T. pronubana*: both are fused to form one long slit-like aperture. There is no pronounced forward extension of the 10th abdominal segment as Poulton figures for several Heterocerous pupae: in other words, the posterior boundary of segment 9 is not markedly extended forwards, the 9th segment projecting forwards distinctly from its anterior boundary. The intersegmental lines between segments 8 and 9, and again between segments 9 and 10, are not continuous across the ventral surface of the pupa.

Emergence.

Prior to the emergence of the moth the pupa wriggles itself out of its shelter by violently agitating its abdomen. In the pupa of *T. pronubana* abdominal segments 4, 5, 6 and 7 are movable in the male and 4, 5, and 6 in the female. According to Chapman (1893) this grouping of movable abdominal segments is found throughout the family Tortricidae. It is by the agitation of those segments and with the assistance of the backwardly directed spines on its dorsal surface that the pupa moves from its pupal shelter, remaining attached by the hooks on the cremaster to the silk which formerly had entirely surrounded it. The head and thoracic regions then split and the moth emerges, leaving the empty pupal case projecting from the leaves among which it had been protected. The moth, on first emerging, remains close to the pupal case till its wings expand. Pairing takes place soon after emergence.

Duration of the Pupal Stage.

The duration of the pupal stage was found to vary to a great extent. The minimum period recorded was 11 days while the maximum was 45 days. It was found that the pupal period of larvae of the winter brood was longer than that of larvae of the summer brood. Pupae kept under similar conditions—on the same plant and in the same greenhouse and which had commenced the pupal stage on the same day—opened after periods differing by as many as six days from one another.

Table IV shows the comparative duration of the pupal period of the summer and winter broods together with the variation that was observed in the case of pupae reared in Edinburgh and at Kew.

Table IV.

Pupation	Emergence	Duration of pupal period (days)
1921—*Summer brood* (Kew)		
7th Sept.	22nd Sept.	16
9th „	19th „	11
14th „	30th „	17
28th Aug.	9th „	13
1922—*Winter brood* (Edinburgh)		
17th March	16th April	31
23rd „	6th May	45
30th „	16th „	18
1923—*Winter brood* (Kew)		
18th April	16th May	28
23rd „	20th „	27
23rd „	18th „	26
25th „	17th „	22
8th May	7th June	30
16th „	11th „	26

Summary of Life-History.

There are two main broods of *T. pronubana* in the year—a summer brood which emerges from eggs laid in spring and a winter brood which emerges from eggs laid in autumn. During winter (October–March), 1921–2, larvae, which emerged in September at Kew, were transferred to Dr R. Stewart MacDougall's laboratory, Edinburgh, where the temperature corresponded to that at Kew (50–60° F.). The larvae continued to feed slowly throughout the winter months and showed no signs of hibernating. The first pupa was obtained from these larvae in March. The larval stage of the winter brood was thus prolonged to 5–7 months. The results of the observations in Edinburgh were confirmed in the laboratory and green-houses at Kew during winter, 1922–3. In one series of experiments where larvae were reared throughout the winter, under green-house conditions, the minimum period of larval life of the winter brood was 33 weeks.

The pupal period of the winter brood varies from 1½–7 weeks (Table IV). Moths of the winter brood appear in April and continue to emerge throughout the spring.

The following notes on the summer brood are based on observations made at Kew. The first eggs of the summer brood are laid from the middle of April onwards. In the laboratory the minimum period of larval life of this brood was 68 days—10 weeks—and the duration of the pupal period averaged 15 days. Moths of the summer brood emerge from the beginning of August and onwards when they deposit the first eggs of the winter brood.

There is marked overlapping of broods. Larvae emerging from eggs laid late in spring do not mature in the autumn but feed along with young larvae of the winter brood derived from eggs laid in the autumn. Further, whereas it was found that the majority of larvae emerging in the beginning of June, pupated towards the end of August, a small number, on the other hand, continued in the larval stage throughout the winter and pupated the following spring. It is evident, therefore, that under glass, all stages of larvae can be found throughout the year.

Mr J. H. Durrant has informed the writer that moths were seen by him near London flying around privet hedges in the open during November, which further confirms the overlapping of summer and winter broods.

The observations and experiments conducted at Kew confirm those made by Adkin (1907). This worker also draws attention to the prolongation of the larval life of the species and the occurrence of "two fairly well defined broods in the year." It would appear that under glass, at the temperatures at which *T. pronubana* is found to be troublesome at Kew (50–60° F.), the two annual broods are also fairly well defined, the moths issuing in numbers in early spring and late summer. As in the open, the larval life is prolonged during the winter months.

Table V.

Stage	Autumn brood	Summer brood
Egg-masses	From Middle of August	April–June
Larvae	End of August–March, April	Majority feed April–August—a few over winter
Pupae	End of March, April and May	August onwards
Adults	Beginning of April onwards	Majority end of August to September. Others continue to emerge even into November

The following table (VI) gives a summary of the observations at Kew on the larvae and moths derived from single egg-masses of summer and winter broods respectively.

Table VI.

SUMMER BROOD.

Larvae reared on Carnations in laboratory.

Oviposition 27th–28th May.
Hatching 8th–9th June.
Pupation 1st pupa on 14th August.
Emergence 17 pupae all opened by beginning of October.
Duration of egg stage = 13 days.
„ larval „ = 68 „
(„ pupal „ = 15–17 days from 1921 observations.)
Minimum period egg-adult = 13–14 weeks.
Average temperature during period = 63° F.

WINTER BROOD.

Larvae reared on Carnation plants in green-house.

Oviposition —
Hatching 30th August.
Pupation *1st pupa 18th April—majority between 1st and 12th May.
Emergence 1st adult 16th May.
Duration of egg stage = —
„ larval „ = 33 weeks (minimum)—average 35–36 weeks.
„ pupal „ = 28 days (1st pupa)—average 27 days.
Minimum period from hatching of egg to adult = 37 weeks.

* Two larvae have still not pupated on 26th June.

Table VII gives a summary of the duration of larval instars of the summer brood. The larvae were all derived from a single egg-mass and were reared on carnations in the laboratory, the temperature of which corresponded to that of the carnation propagating house. Owing to the habit of the larvae in the later instars of wandering about on the host plants it was found impossible to follow individuals from stage to stage: as far as possible, however, this was done. As has previously been stated (p. 406) great variation occurred in the duration of the instars.

Table VII.

Emerged	Dates of moults (1922)							Lengths of instars in days						
	1	2	3	4	5	6	7	1	2	3	4	5	6	7
8. vi.	18. vi.	27-28. vi.	6. vii. 7. vii. and onwards	18. vii. 19. vii.	27. vii *30. vii.	*9. viii.	14. viii. 15. viii. and onwards							
				26. vii. and onwards	*1. viii. and onwards	*14. viii. 22. viii.	during Sept.	10-11	10-11	9-12 and over	8-20 and over	9-10 and over	10-26	14-22

Average minimum duration of instar = 9-10 days.
Average day temperature during larval life = 63·5° F.

* Denotes same larva followed throughout both instars.

III. ECONOMIC IMPORTANCE.

HOST PLANTS.

The first recorded host plant of *T. pronubana* in the open in Britain is a species of Euonymus (Adkin, 1905). The species is now found in abundance in certain localities in the South of England feeding on euonymus and privet. At the Royal Botanic Gardens, Kew, it is present in large numbers, also on ivy and on laurel. It is only within the last three or four years, however, that the moth has made its appearance under glass at Kew and there the larvae are found feeding on all kinds of plants at temperatures varying between 50 and 60° F. A list of the plants on which the larva is found under glass is given in the Appendix, and, although not exhaustive, it illustrates the polyphagous habit of the species and shows the wide range of plants, embracing many Natural Orders, on which the larva thrives.

Through the kindness of the director, Mr Chittenden, a visit was paid to the Royal Horticultural Society's gardens at Wisley in August, 1922, and again in August, 1923. Mr G. Fox-Wilson the entomologist stated that he had never been troubled with *T. pronubana.* On both occasions a careful examination of the carnations and other plants under glass was made and it is interesting to note that no trace of *T. pronubana* was found. It is curious that an insect, which has proved so troublesome at Kew, should be entirely absent from Wisley and it is probable that the species occurs in abundance only in certain localities in the South of England.

A thorough examination of the glass-houses at the Royal Botanic Gardens, Edinburgh and at the Botanic Gardens, Glasgow showed that *T. pronubana* did not occur in either locality.

In Edinburgh, however, it may be noted that another Tortrix species was found in large numbers on a specimen of *Araukaria braziliensis* in the Large Palm House. The tree was badly attacked—the larvae not only spinning the leaves together and feeding on them but also boring into the stem and shoots causing exudation of resin, defoliation and death of leaves and shoots. Numbers of larvae were collected and reared in the laboratory at Edinburgh. Moths were obtained from these in the beginning of May and were identified by Mr Durrant of the British Museum as *Batodes angustiorana,* Haw.

INJURY TO PLANTS BY *T. PRONUBANA*.

Signs of Attack.

The first indications of *T. pronubana* attacks are afforded by the spinning habit of the species so characteristic of the family Tortricidae. Immediately after emerging the young larvae wander about in search of a suitable place to commence feeding and having found such a place they at once begin to spin a shelter for themselves. Where the plants attacked possess small leaves, *e.g. Cytisus fragrans* and *Pimelea* sp. the presence of the young larvae is more noticeable than in plants possessing larger and coarser leaves, *e.g. Dianthus* spp. In the former case several leaves are soon spun together while in the latter type of plant the spinning of the young larvae is not easily seen since they usually spin at the tips of leaves or in leaf axils. Consequently, the characteristic knots of twisted spun-together leaves, so obvious in small leaved plants, are not at first present. As the larva grows, however, they become more apparent.

Of the various host plants cited in the Appendix, *Dianthus* spp., *Cytisus fragrans*, *Acacia* spp. (in particular *A. longifolia* var. *mucronata*) and *Chorizema ilicifolium* are the most severely attacked. In all cases it was found that the larva feeds on the leaves and foliage, particularly at the young growing tips of the plants attacked. On carnations the presence of young stage larvae is difficult to detect.

In addition to feeding on the leaves it is common to find larvae attacking unopened buds. This is particularly noticeable in carnations and *Pimelea* sp. A small hole (Fig. 16) in the side of the bud is the only indication of infestation, the bud in all other respects appearing quite normal. Within such a bud, however, a larva is present, feeding on the flower within and thus entirely destroying it. In late autumn, November, the flower buds of *Pimelea* sp. were badly attacked.

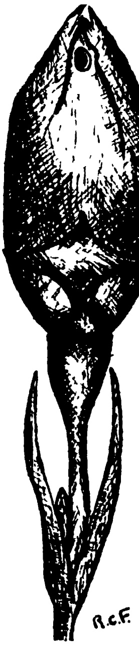


Fig. 16. Carnation bud (enlarged) containing larva of *T. pronubana*.

Note hole, the only sign of attack. This is the typical appearance of attacked buds of different species of plants.

The larva does not confine its feeding to leaves and unopened buds but also feeds on flowers, *e.g.* flowers of Carnations, Cytisus and Acacias are frequently found with larvae feeding upon them. A carnation flower attacked by 1st and 2nd stage larvae has its petals perforated with small holes resulting from the feeding of the young caterpillars.

Injury Resulting from Attacks.

The damage done to the host plants is due to the destruction of the leaf tissue and consequent reduction of leaf area. In the laboratory where young carnations were used for rearing the larvae it was found that a perfectly healthy plant was killed 6–8 weeks after infection with a number of larvae. The photographs (Plate XV) show three plants, before, during, and after an attack by *T. pronubana.*

In the glass-houses at Kew, however, the larvae are not present in sufficient numbers to cause the death of the plants; if, on the other hand, they were allowed to increase in numbers, there would be a grave risk of causing the death of the smaller plants attacked. Buds and flowers are alike destroyed and thus again injury of a different nature done to the plant.

Quite apart from the damage done to the health of the host plant, the presence of *T. pronubana* larvae tends to mar its beauty, causing bunches of twisted, shrivelled and withering leaves to appear (Plate XV, plant B), and flowers to become distorted and unsightly with the petals eaten and spun together. Such harmful effects are of considerable importance anywhere, and particularly in such places as the Royal Botanic Gardens, Kew, where it is essential to maintain the beauty as well as the health of the plants.

MODE OF DISPERSAL.

Reference has already been made (p. 404) to the habit of the 1st stage larvae immediately after emerging of wandering some distance before settling down to feed. In a green-house, therefore, it is obvious that infection from plant to plant at this stage is a simple matter, especially when the polyphagous habit of the larva is remembered. Further, the characteristic habit of the young larva of hanging in festoons from the leaf tips is of importance with regard to the spread of the pest from one plant to another. The slightest draught waves these festoons to and fro so that they often come in contact with neighbouring plants. In a green-house there is not the same possibility of wind playing such a large part in the distribution of young larvae as in the open. It is frequent, however, to find quite a distinct draught in a green-house, especially on a hot summer's day when the "lights" are open.

The adult moths flying throughout the green-house obviously play an important part in dispersal since the females lay their eggs on practically any plant and also on the glass sides and roof of the house. It is apparent that the pest could be eradicated from any given glass-house if means were taken to prevent the entrance of moths from the open. The use of insect-proof screens of wire gauze, or, less expensive, of muslin which could be fitted to cover all openings when windows are open, would effectively prevent moths entering or leaving a house. In this way, each house could be separately treated for the pest and eventually it would be entirely eradicated. The screens, however, would require to be used permanently since the species breeds freely in the open on privet, euonymus and ivy.

A further means of dispersal is found in the introduction of infected plants into houses formerly quite "clean." In Botanic gardens it is the usual practice to transfer plants from propagating houses to the larger green-houses. Thus it is obvious that infected plants may be conveyed from house to house and ideal conditions provided for the spread of the pest. This means of spread could be remedied by having all plants carefully examined before transferring them from house to house.

IV. CONTROL.

The following series of experiments was conducted with a view to ascertain the most practical and effective methods of controlling *T. pronubana* under green-house conditions. A study of the life-history has shown that such experiments should be devised to deal with the young larvae immediately after emergence.

SPRAYING EXPERIMENTS.

A. *Effect of a Nicotine Spray on Newly-Emerged Larvae.*

Exp. 1.

Nicotine spray—Commercial XL–All: used in the proportion of 1 part to 30 parts water.

May 23*rd*. Plants sprayed; larvae on plants 1st stage—48 hours old.

May 25*th*. Plants examined—results as shown in Table VIII.

Table VIII.

Plant	Larvae dead	Larvae alive	Totals	% killed
A. (Carnation)	34	12	46	73·9
B. („)	14	7	21	66·6
C. („) (Control, unsprayed)	—	7 (All found)	7	—

With a few exceptions the larvae not killed were in places which the spray had not reached, *i.e.* protected in the axils of leaves and in several instances sufficiently protected from the spray by a silken shelter already partly spun.

Where the spray reached the larvae death resulted, but it is apparent that it is not possible to reach all larvae even 48 hours after emergence.

Exp. 2.

According to Gibson and Ross (1922) the following nicotine and sulphur spray was used with excellent results in green-houses in Eastern Canada against the Green-house Leaf-Tyer:

Phlyctaenia ferrugalis, Hb.

Soluble sulphur	1 ounce by weight.
Nicotine sulphate (40 %)	1 fluid ounce.
Water	6 gallons.

It was decided to use this spray in the above proportions on an experimental scale against 2nd and 3rd stage larvae of *T. pronubana*. The following are the results obtained:

July 6th. Three carnation plants A, B, C on which 1st, 2nd and 3rd stage larvae were present, since emergence, all thoroughly sprayed, as above.

July 7th. Plants examined.

A.	Larvae dead = 6	Larvae alive = 36
B.	„ = 2	„ = 17
C.	„ = 2	„ = 10
	Totals 10	63

Percentage killed = 16.

In all cases the larvae killed were 1st stage and so not protected to the same extent by spun-together leaves as the older larvae. It is evident that the small percentage destroyed is due to the fact that the spray did not reach the older larvae.

B. *Effect of Nicotine Sprays on Egg-Masses.*

Exp. 3.

Plants—carnations—each with an egg-mass on their leaves thoroughly sprayed with XL-All (1 in 30): egg-masses in all cases just about to hatch.

Table IX.

Plant	Sprayed	Larvae emerged	Remarks
A.	25th June	—	Egg-mass did not hatch: eggs shrivelled up
B.	28th June	30th June	All eggs hatched: but two dead larvae found beside empty egg-shells
C. Control and unsprayed		26th June	All eggs hatched
Four isolated egg-masses	30th June	First mass hatched July 3rd	By July 9th majority of eggs in sprayed and unsprayed masses had hatched: out of all larvae four or five died on emerging: % dead, negligible
Four isolated egg-masses	Control and unsprayed		

Exp. 4.

Egg-masses sprayed with nicotine and sulphur spray (see above).

June 25*th*. Mass A. Of approximately 60 eggs, sprayed.
„ B. „ 40 „
„ C. Control and unsprayed.

June 26*th*. Mass C. All eggs hatched.

June 27*th*. Mass A. Majority of eggs hatched; 1 dead larva beside mass.

Mass B. Majority of eggs hatched.

June 28*th*. Mass A. All eggs hatched except four which were not yet mature.

Mass B. Two dead larvae beside mass; several eggs not yet hatched.

July 2*nd*. Remaining eggs hatched in both masses.

In the above experiments nicotine and nicotine and sulphur sprays in the strengths used did not prevent the hatching of the egg-masses. In one case only (Exp. 3, Mass A) did a sprayed egg-mass not hatch. It is likely that the death of this mass was due to some other cause since all other masses sprayed under the same conditions hatched.

The larvae, on emerging, settled down to feed, as usual, on the leaves of the plants, the nicotine by this time having apparently no effect on the young caterpillars.

In no case did the plants used in the above experiments with nicotine sprays suffer in the least.

C. *Effect of Arsenate of Lead Spray on Larvae.*

In the following experiments Strawson-Swift Lead Arsenate Paste was used in the proportion of 6 lbs. to 50 gallons of water. This strength of spray is recommended by Lloyd (1920) for the control of *Hadena oleracea.*

Exp. 5.

June 6*th.* Plant A. With an egg-mass of approximately 50 eggs just about to hatch; thoroughly sprayed as above.

June 8*th.* Egg-mass found hatched.

June 9*th.* Eight larvae found; many others wandering over plant and beginning to feed.

June 11*th.* Seventeen dead larvae; still a few feeding.

June 13*th.* Two dead larvae; no others visible.

Total larvae killed = 19 and plant clean.

In the control plant all leading shoots infected with larvae from an egg-mass which hatched on June 7th.

June 8*th.* Plant B. Infected with 1st stage larvae and then sprayed as above.

Plant C. Sprayed; eggs about to hatch.

June 9*th.* B. 1 dead larva.
C. Egg-mass just hatched.
June 11*th.* B. 1 dead larva.
C. 2 dead larvae.
June 13*th.* B. 2 ,,
C. 8 ,,

Both plants sprayed again with freshly-made spray: suspected that first spray on June 8th was not satisfactorily mixed.

June 15*th.* B. 10 dead larvae; several living larvae feeding on under side of leaves where spray had not reached.
C. 20 dead larvae.
June 18*th.* B. 6 ,,
C. 8 ,,
June 21*st.* B. 1 dead larva.
C. 1 ,,
June 25*th.* B. 2 dead larvae; none alive.
C. 0 dead larva; 1 alive.

Experiment closed.

Total larvae killed B. 23
,, ,, C. 39
Total 62

Although it is not possible in this experiment to estimate the actual percentage of larvae destroyed since many wandered away from the plants when they first emerged, yet it is apparent that 18 days after the hatching of the egg-masses the plants were found to be free from larvae.

The control plant at the end of the same period was showing signs of severe attack, the leaves being spun together and the tips of many withering where the young larvae had been feeding.

Effect of Lead Arsenate on Plants.

Carnation plants were used in the above experiments and they did not suffer in the least from the spray: the only effect of spraying being the pale whitish colour imparted to the leaves by the deposit of lead arsenate left as the spray dried.

FUMIGATION WITH HYDROCYANIC ACID GAS.

With a view to ascertaining whether hydrocyanic acid gas was of any value for destroying the larvae of *T. pronubana* a series of experiments were conducted in a small green-house (cubic contents 828 cu. ft.) specially prepared for the purpose. The following is a summary of the experiments conducted. Valuable information regarding fumigation with hydrocyanic acid gas and the quantities recommended were obtained from Bulletin No. 513 (Sasscer and Borden, 1917) of the U.S. Department of Agriculture.

Exp. 1. 14th August.

Strength of cyanide used = ¼ oz. per 1000 cu. ft.
Exposure = 1¼ hours.
Temperature during exp. = 62° F.

Table X.

Table of Results.

Plant	Alive—before exposure	Dead after exposure	Remarks
Carnation	12 (3rd–7th stages)	0	Only one carnation plant showed any sign of burning of leaves
„	7 „	0	
„	4 „	0	
„	3 „	0	
„	2 „	0	
Coleonema albuma	4 (2nd–4th stages)	0	Slight burning of leaves
Rhododendron Edgeworthii	1 (7th stage)	0	
Lhotskya ericoides	1 „ „	0	
Acacia Drummondii	4 { 3rd „ 14th „	2*	This plant was nearest jar containing cyanide
Acacia pulchella	5 various stages	1*	

% of larvae killed = 7.

* In the light of subsequent experiments, with stronger doses of cyanide, it is doubtful whether the death of these larvae resulted from hydrocyanic acid gas.

Exp. 2. 15th August.

Plants as in Exp. 1 with the surviving larvae.

Strength of cyanide = ½ oz. per 1000 cu. ft.
Exposure = 2 hours.
Temperature = 67° F.
Percentage of larvae killed = nil.

Exp. 3. 25th August.

Plants and larvae as in previous experiment.

Strength of cyanide = 1 oz. per 1000 cu. ft. (requisite quantity divided between two jars so as to ensure better distribution of gas).

Exposure = 2½ hours.
Percentage of larvae killed = nil.

Exp. 4. 26th August.

Plants and larvae as before.

Strength of cyanide = ½ oz. per 1000 cu. ft. but in two jars.
Exposure = 12½ hours.
Percentage of larvae killed = nil.

During the above experiments larvae were also placed in the green-house in petrie dishes covered with muslin and thus exposed to the gas. In no case, however, were larvae killed.

It may be concluded that fumigation of plants infected with larvae of *T. pronubana* is of no value as a control measure.

FURTHER EXPERIMENTS.

Pyrethrum powder burnt in a large cage in which had been placed a plant attacked by larvae of *T. pronubana* at various stages was found to have no effect whatever on the caterpillars.

Fumigation with tobacco fumes as is regularly done at Kew to destroy green-fly had also no effect on the larvae: nor were moths destroyed which had been confined in a cage—two sides of which were made of wire gauze—the cage being placed in a green-house during fumigation with tobacco.

CONCLUSIONS DRAWN FROM CONTROL EXPERIMENTS.

The control of *T. pronubana* is a difficult problem and the brief experiments cited above are sufficient to show that it is impossible to outline complete and successful control of the insect without a further series of experiments conducted on a large scale under green-house conditions.

It is, however, evident that nicotine and lead arsenate sprays are both effective if applied immediately after the larvae have emerged and before they have begun to spin up. The chief difficulty experienced was that of reaching the young larvae with the nicotine spray, while in the same way it was not easy to coat the entire plant with lead arsenate. Unavoidably many leaf surfaces (those spun together) were not covered with the poison. The older the larvae the more serious was this difficulty. Both upper and lower surfaces of the leaves require to be sprayed. It is apparent that systematic and continued spraying with a nicotine spray from the time the first eggs of the year are expected to hatch (end of March) would account for large numbers of young larvae. Owing to the marked overlapping of broods in this species, the spraying must be carried on at intervals (say once per week, plants thoroughly sprayed) throughout spring, summer and autumn.

While the results of the arsenical spray are less rapid and less apparent than those of the contact insecticide, yet, since the larvae wander during feeding it is obvious that if the plants be periodically sprayed the death of many will eventually result as has been shown in the above experiments.

Gibson and Ross (1922) found a contact spray successful for the control of *Phlyctaenia ferrugalis* and so it would also appear that a nicotine spray is to be recommended in the case of *T. pronubana.* In the experiments at Kew it was found that spraying with any insecticide is useless unless the young larvae (1st and 2nd stages) are tackled: the spinning habit of the species makes it impossible to reach the larvae or the leaf surfaces on which they are feeding at later stages.

Fumigation with hydrocyanic acid gas is of no use in dealing with the pest. Molinas (1914) found that fumigation with nicotine gave no results and after experimenting with sulphuric acid and hydrocyanic acid gas finally abandoned any type of fumigation as successful control measures.

At Kew the larvae have been destroyed and their numbers successfully reduced by hand-picking. While this is a comparatively easy matter when the larvae are half to full grown, the younger stages are not easily seen and are thus usually passed over. Systematic destruction of larvae, pupae and egg-masses—the latter being frequently found on the "lights" of green-houses—are all simple, practical means of reducing the numbers of the pest under glass. It is, however, futile to expect to eradicate entirely *T. pronubana* from green-houses in a locality such as Kew, where the species is found abundantly in the open in their immediate vicinity. Mention has already been made (p. 431) of the suggested use of muslin or wire gauze screens to fit all openings in the green-houses and so prevent entrance and exit of adults. If this be done and systematic spraying and examination of plants be carried out before transferring from house to house, the pest would, as far as can be seen at present, be eradicated from infected green-houses.

In conclusion, it may be remarked that at Kew, *T. pronubana* has not proved so troublesome during 1923 as during 1921 and 1922 when this work was first begun. Hand-picking has been carried out with success throughout the period.

V. PARASITES OF *T. PRONUBANA*.

Millière (1859) describing the life-history of *T. pronubana* on the Continent, states that his observations showed the species to be parasitised to an extent of 75 per cent. by a Tachinid *Mornia bigoti*, Robineau.

Adkin (1907), working in this country, in his first observations did not obtain a single parasite. During summer, 1908, however, he reared several specimens of a Tachinid—*Zenillia roseana*, B. and B.—from larvae and pupae of *T. pronubana*. The writer has recently been in communication with Adkin on this question of parasites and with his permission quotes the following extract from a letter he has received and in which Adkin records the Ichneumon *Oedematopsis scabricula*, Grav. as a parasite of *T. pronubana*:

"...in 1907 I turned a lot of *T. pronubana* eggs out on an euonymus bush in my garden at Lewisham. In the spring of 1908 I searched the bush for larvae but could find only two and each of them had a parasitic larva attached to it. I cannot say whether there had been more of the Tortrix larvae and that they had been killed by the parasites or not, but these two were eventually sucked dry by them. I sent the parasites which I reared from these two to Claude Morley and he identified them as the Ichneumon, *Oedematopsis scabricula*, Grav. The parasites attach themselves to their hosts externally and the best description I can give of their appearance is that they are curled between the 2nd and 3rd segments or perhaps 3rd and 4th. Larva found with parasite attached—April 12th.

Parasite emerged—May 29th and June 9th."

An Hymenopterous larva was found feeding on a larva of *pronubana* just as Adkin describes above on April 13th, 1923 on a privet hedge at Richmond. Unfortunately the parasite did not attain maturity but it is probable that this was the same species of Ichneumon as Adkin obtained.

In the observations made under glass at Kew several specimens of two (probably) Hymenopterous parasites of *pronubana* have been obtained but never have any Tachinid parasites been found. In August and September, 1921, three Ichneumons issued from pupae of *pronubana*—the only parasites obtained during the summer of that year. In 1922 no parasites were found, although a thorough search was made in the houses at Kew and 30 or 40 pupae were collected from all of which moths emerged. In spring, 1923, eight Hymenopterous cocoons were collected from plants in the green-houses; beside each cocoon a shrivelled larval skin was found. During April and May the adult parasites emerged and an experiment was commenced to follow out their life-history. Owing to lack of material, however, this had, for the present, to be abandoned. The parasites obtained have been submitted to Dr Waterston of the British Museum and he has promised to identify them. At present, however, the names of the species have not been received. It will be interesting to see whether they are the same as those reared in the open by Adkin. Owing to the extremely small number of parasites obtained at Kew it is quite impossible to give any figures representing the percentage of parasitism.

It would appear that, up to the present, *T. pronubana* is singularly free from parasites in Britain. Since the species is a native of foreign shores and is not indigenous to this country it has evidently been introduced without the parasites which attack it so successfully and, according to Millière, in such numbers in its native habitat. Hence as a natural means of control, parasitism must be regarded at present as of little importance, especially since parasitised larvae, and therefore parasites, as well as unparasitised larvae are destroyed by hand-picking.

VI. *TORTRIX PRONUBANA* IN RELATION TO DYAR'S HYPOTHESIS.

Dyar (1890) in a paper on the "Number of Moults of Lepidopterous Larvae" establishes an hypothesis which he has found to hold good for all the species of Lepidopterous larvae he has examined. His hypothesis states that "the widths of the head of a larva in its successive stages follow a regular geometrical progression and if, in examining the measurements of heads taken in following out a life-history, any deviation from the calculated progression is shown, it is evidence that an error has been committed or that the larva has behaved in an abnormal manner." Dyar selected the head of the larva for measurement "as the part not subject to growth during the stage, and its width as the most convenient measurement to take."

He gives in this paper a list of species which shows the actual and calculated larval head measurements together with the ratio existing between the measurements of successive larval heads. The following are a few examples to explain further Dyar's hypothesis. All measurements are in millimetres and are correct to within ·1 mm.

Species with Five Larval Stages.

Papilio cresphontes, Cram.		
	Calculated	·7, 1·1, 1·6, 2·3, 3·4
	Ratio	·66
	Found	·6, 1·1, 1·6, 2·2, 3·4
Datana palmii, Bent.		
	Calculated	·5, ·9, 1·5, 2·7, 4·6
	Ratio	·58
	Found	·5, ·9, 1·6, 2·7, 4·6

Species with Seven Larval Stages.

Halisidota harrisii, Walsh.		
	Calculated	·4, ·6, ·8, 1·2, 1·7, 2·4, 3·5
	Ratio	·7
	Found	·4, ·6, ·9, 1·4, 1·6, 2·3, 3·5 ·4, ·6, — 1·3, 1·7, 2·6, 3·6

It is probable that the figures Dyar gives are averages selected from a long series of measurements to illustrate the remarkable similarity between the measurements calculated and those actually found. In the figures given in Table XI for *T. pronubana* all measurements are given and it is thus apparent that whereas the general law holds, nevertheless there are considerable variations between measurements of similar stages.

The principle is of considerable importance when viewed from the point of establishing the number of moults for any given species and hints at the possibility of ascertaining the causes of variation in a life-history.

During summer, 1922, whilst conducting experimental work on the biology of *T. pronubana*, it was decided to investigate the larval stages and number of moults on the lines of Dyar's hypothesis. So far as has been observed it was found that growth in the species agreed with the results which Dyar found for other Lepidopterous larvae, namely, that the widths of the head of a larva in its successive stages follow a regular geometrical progression.

In the experiments at Kew the procedure was as follows: batches of larvae were reared on carnation plants and as the different moults occurred the cast heads found after each moult were measured by means of an eye-piece micrometer. At the same time the breadth of the head of the newly-moulted larva was also obtained and in this way a series of measurements of larval heads and cast head capsules got for each instar. It was found impossible to follow an individual larva throughout its life because of laboratory conditions where numbers of larvae were kept together on a limited number of host plants and also since larvae, when disturbed, moved to another part of the plant so that it was not always possible to obtain with certainty the same individual that had been measured in preceding instars.

Measurements of larval heads were taken at the broadest part; it was found that if cast heads were mounted in balsam on glass slides before measurement the weight of the cover glass caused the head to split and give too large a reading. This was especially the case in the later instars. Accordingly it was found most satisfactory to measure all cast head capsules without mounting, merely placing them dry on a microscopic slide and measuring in the usual manner with the use of an eye-piece micrometer.

The breadth of larval heads of similar stages was found to vary slightly as can be seen in Table XI given below. The variation, however, was not sufficient to upset Dyar's hypothesis. The ratio between the breadth of larval heads of *T. pronubana* in any two successive instars was 1·37. For example, the average breadth of a 1st stage larval head was ·24 mm. and that of a 2nd stage larval head ·33 mm. The ratio ·33 : ·24 is thus 1·37. Having obtained this ratio it is possible to calculate the size of the larval head of any other instar. Further, it enables one to determine the exact stage of growth of any given larva found at random in the green-houses or elsewhere.

During 1922 the summer brood larvae underwent seven moults, the pupa appearing after the seventh moult. In October an interesting observation was made: the cast head of a recently-moulted larva of the summer brood was compared with that of the larva itself and it was found that, while the cast head (·68 mm. broad) indicated 4th stage, the head of the larva itself indicated 6th stage (1·1 mm. broad). It is thus apparent that the 5th stage of this caterpillar had been entirely omitted and at moult IV instead of getting a 5th stage larva a larva corresponding to a true 6th stage was obtained. It must be borne in mind that the duration of the first four instars of the life of this larva had extended over an unusually long period. The result of this appears to have been elimination of the usual fifth moult and the production of a 6th stage larva at moult IV. Further similar cases of delayed development resulting in the omission of a moult (omission of more than one moult was never recorded) were observed during autumn, 1922.

The conclusion may thus be drawn that, whereas a larva may prolong its life to a considerable extent, in so doing it undergoes fewer moults than a larva undergoing more rapid normal development. This would seem to be the direct result of the slow growth itself which would appear to render frequent moults unnecessary. It forms evidence to show, as has been frequently stated, that changes of skin or moults are the result of growth.

Table XI.

Head Measurements of Larvae of T. pronubana, *Hb. in Successive Instars.*

Stage	Micrometer measurements of larval heads (mm.)	Micrometer measurements of cast head capsules (mm.)	Calculated measurements (mm.)
1st*	·22, ·23, **·24**, **·24**, **·24**, ·26	·31, ·31, ·31, ·32	·24 = ·24
2nd*	·30, ·31, **·33**, **·33**, **·33**	·41, ·42, ·42, ·43, ·44, ·44	·24 × ·37 = ·33
3rd	**·44**, **·46**, **·46**, **·46**, ·48, ·51, ·55	·46, ·58, ·66*, ·65*, ·67*	·33 × ·37 = ·45
4th	**·62**, **·62**, **·62**, ·63, ·63, ·65, ·67	·60, ·60, ·62, ·62, ·65, ·68, ·73	·45 × ·37 = ·61
5th	·78 (?), **·82**, **·84**, ·87, ·88	·80, ·81, ·84	·61 × ·37 = ·83
6th	·94, ·97, 1·0, **1·1**, **1·1**, **1·1**, 1·2	·90, ·97, 1·04, 1·1, 1·1	·83 × ·37 = 1·13
7th	1·2, 1·3, 1·4, **1·5**, **1·5**, **1·5**	1·5, 1·2	1·13 × 1·37 = 1·54

* Denotes cast head capsules mounted in balsam before measurement—hence large readings.

Ratio, calculated, = 1·37.

SUMMARY.

The moth, *Tortrix pronubana*, Hb., has lately proved troublesome in the glass-houses at the Royal Botanic Gardens, Kew. Its life-history has been studied and shows two main broods in the year. The periods of duration of the various larval stages and of the pupal stage have been studied in detail.

A description of the external morphology and chaetotaxy of the larva is given. Setal maps are given showing the distribution of the setae on the thoracic and abdominal segments of the 1st stage and full-grown larvae. The chaetotaxy of the larval stages is similar except in the 1st stage. The growth of the larva throughout its various stages, seven in number (normally six moults), conforms with Dyar's hypothesis that "the widths of the head of a larva in its successive stages follow a regular geometrical progression and if, in examining the measurements of heads taken in following out a life-history, any deviation from the calculated progression is shown, it is evidence that an error has been committed or that the larva has behaved in an abnormal manner."

The chaetotaxy of the abdominal segments of the pupa is described and sex characters are noted.

A series of experiments on the control of *T. pronubana* under glass shows that spraying with nicotine or lead arsenate is effective if applied before and immediately after the eggs hatch. It is essential that the spray should be applied before the larvae commence to spin their webs. Destruction of the larvae by hand-picking is effective but the minute 1st and 2nd stage larvae are easily overlooked. Hand-picking combined with regular spraying is to be recommended as the best method of control.

Fumigation with hydrocyanic acid gas was found ineffective against larvae.

The screening of windows and ventilators with gauze or muslin is recommended as a check on the infestation of clean houses.

Two Hymenopterous parasites, not yet identified, were bred from larvae and pupae of *T. pronubana* but the percentage of parasitism was extremely low.

A list of host plants of *T. pronubana* under glass and in the open is given in an appendix.

In conclusion the writer wishes to express his indebtedness to Dr J. W. Munro for his advice and encouragement during this work which was carried out under his supervision. Thanks are due to the Director of the Royal Botanic Gardens, Kew, for the many facilities which were so willingly granted for experimental work and for general observations in the green-houses.

APPENDIX.

Host plants of *T. pronubana*, Hb. under Glass at Kew.

* *Denotes plants on which larvae are most abundant.*

- **Acacia, L.** . . . . **Leguminosae**
  - **A. armata, R. Br.**
  - **A. baileyana, F. Muell.**
  - **A. cultriformis, A. Cunn.**
  - **A. Drummondii, Benth.**
  - ***A. longifolia, Willd.**
    - **Var. mucronata**
  - **A. neriifolia, A. Cunn.**
  - **A. platyptera, Lindl.**
  - **A. pravissima, F. Muell.**
  - **A. pulchella, R. Br.**
  - **A. riceana, Hernel.**

Agapetes, G. Don	Vacciniaceae
A. Moorei	
Albizzia, Durazz	Leguminosae
A. Julibrissin, Durazz	
Asparagus, Tourn..	Liliaceae
A. sprengeri, Regel.	
Boronia, Sm.	Rutaceae
B. elatior, Barth.	
Bucklandia, R. Br.	Hamamelideae
B. populnea, R. Br.	
Callistemon, R. Br.	Myrtaceae
C. Cunninghamii, Hort.	
C. salignus, DC.	
Calythrix, Labill.	Myrtaceae
C. tetragona, Labill.	
Cestrum, L.	Solanaceae
C. aurantiacum, Lindl.	
(Larvae yellowish in colour on this plant.)	
Chorizema, Labill.	Leguminosae
*C. ilicifolium, Labill.	
Clianthus, Banks & Sol.	Leguminosae
Species not determined.	
Coleonema, Barth. & Wendl.	Rutaceae
C. albuma, B. & W.	
*Coronilla, L.	Leguminosae
C. glauca, L.	
Var. variegata	
Cytisus, L.	Leguminosae
*C. fragrans, Lam.	
C. filipes, B. & W.	
Daphne, L.	Thymelaeaceae
D. odora, Thumb.	
*Dianthus, L.	Caryophyllaceae
Various species	
Erica, L.	Ericaceae
E. bowieana	
Eriostemon, Sm.	Rutaceae
E. buxifolius, Sm.	
E. scaber, Paxt.	
Geranium, L.	Geraniaceae
Species not determined	
Grevillea, R. Br.	Proteaceae
G. alpina, Lindl.	
G. oleoides, Sieber.	
G. ornithopoda, Meissn.	
G. punicea, R. Br.	
G. robusta, A. Cunn.	
G. thelemanniana, Hugel.	
Helichrysum, L.	Compositae
H. rupestre, DC.	
Lhotskya, Schan	Myrtaceae
L. ericoides, Schan.	

Mitraria, Cav.	Gesneraceae
M. coccinea, Cav.	
Nandina, Thmb.	Berberideae
*N. domestica, Thb.	
(Larvae markedly yellow on this plant)	
Pimelea, Banks & Sol.	Thymelaeaceae
P. ferruginea, Labill.	
P. Gnidia, Willd.	
Var. pulchella	
P. ligustrina, Labill.	
Poinciana, L.	Leguminosae
P. Gilliesii, Hook	
Psoralea, L.	Leguminosae
P. pinnata, L.	
Rhododendron, L.	Ericaceae
R. Edgeworthii, Hook	
R. formosum, Wall.	
Var. Gibsonii	
R. Oldhamii, Maxim	
R. Schlippenbachii, Maxim.	
Tricuspidaria, R. & P.	Tiliaceae
T. lanceolata, Miq.	

The writer is indebted to Mr J. H. Durrant for the following list of plants from which *T. pronubana*, Hb. has been recorded in the open in Britain and abroad:

Arbutus, L.	Ericaceae
A. unedo, L.	
Aristolochia, Tourn.	Aristolochiaceae
Species not determined	
Asphodelus, L.	Liliaceae
A. ramosus, L.	
Cneorum, L.	Simarubaceae
C. tricoccon, L.	
Cytisus, L.	Leguminosae
C. linefolius, Lam.	
Daphne, L.	Thymelaeaceae
D. gnidium, L.	
Digitalis, L.	Scrophulariaceae
D. obscura, L.	
Euonymus, L.	Celastrineae
E. japonicus, L.	
Euphorbia, L.	Euphorbiaceae
Species not determined	
Geranium, L.	Geraniaceae
Species not determined	
Jasminum, L.	Oleaceae
Species not determined	
Ligustrum, L.	Oleaceae
Species not determined	
Lotus, L.	Leguminosae
L. rectus, L. and other species	

Mercurialis, L. M. elliptica, Lam.	Euphorbiaceae
Olea, L. O. europaea, L.	Oleaceae
Passerina, L. Species uncertain	Thymelaeaceae
Pistacia, L. P. Lentiscus, L.	Anacardiaceae
Rhus, L. R. Coriaria, L.	Anacardiaceae
Robinia, L. R. pseudacacia, L.	Leguminosae
Rosmarinus, L. R. officinalis, L.	Labiatae
Rubus, L. Species not determined	Rosaceae
Scabiosa, L. Species not determined	Dipsaceae
Smilax, L. S. aspera, L.	Liliaceae
Solidago, L. Species not determined	Compositae
Thymus, Tourn. T. vulgaris, L.	Labiatae
Viburnum, L. V. tinus, L.	Caprifoliaceae

BIBLIOGRAPHY.

Adkin, R. (1906–7). On the Occurrence of *Tortrix pronubana*, Hb., in Britain. *Proc. S. Lond. Ent. Soc.* 13–17.

—— (1907–8). Further Notes on *T. pronubana*, Hb., including its Life History in Britain. *Ibid.* 20–26.

—— (1908–9). Note on a Tachinid Parasite of *T. pronubana*. *Ibid.* 86.

Börner, C. (1907). *Hadena secalis*, L. als Roggenschädling. *Arbeit. aus der Kaiserlichen Biologischen Anstalt*, v, 90–97.

Chapman, T. A. (1893). The Structure of Pupae of Heterocerous Lepidoptera. *Trans. Ent. Soc. Lond.*

Dyar, H. G. (1890). Number of Moults of Lepidopterous Larvae. *Psyche*, v, No. 175–6, 420–2.

—— (1893). A Classification of Lepidopterous Larvae. *Ann. New York Acad. Sc.* viii.

—— (1901). A Century of Larval Descriptions. *Ent. Rec.* xiii, 40.

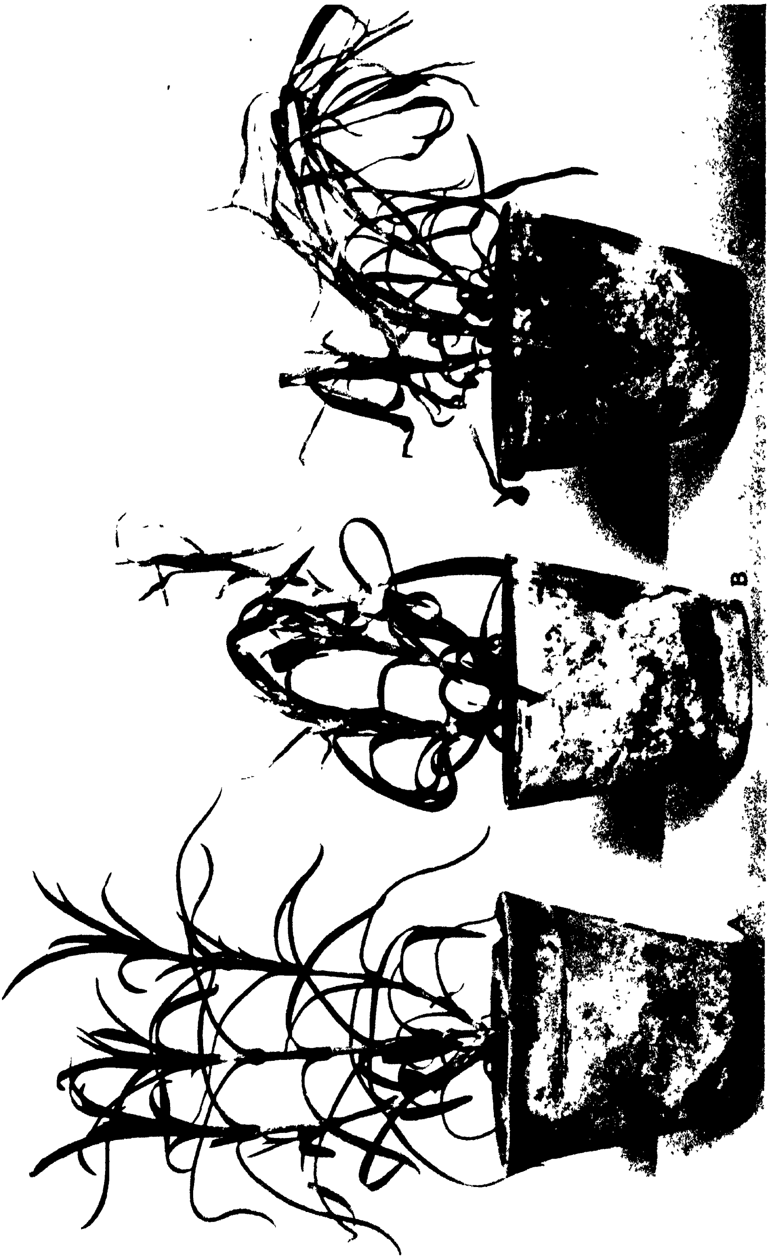
Fracker, S. B. (1915). Classification of Lepidopterous Larvae. *Illinois Biol. Monog.* ii, No. 1.

Gibson, A. and Ross, W. A. (1922). Insects affecting Green-house Plants. *Canad. Dept. Agri. Bull.* 7, N.S. 14.

Hatchett-Jackson, W. (1890). Morphology of the Lepidoptera. *Trans. Linn. Soc. Zool.* (2), v, 143.

Hübner (1797 and 1830). *Samml. Europ. Schm.* vii (Tortr.), Pl. 19, Fig. 121; Pl. 52, Figs. 332–3.

Huie, L. H. (1917). *Eudemis naevana*, Hb., The Holly Tortrix Moth. *Proc. Roy. Phy. Soc. Edin.* xx, Pt 3, 164–78.



FISHER.—The Life-History and Habits of *Tortrix pronubana*. (pp. 395—447.)

LLOYD, LL. (1920). The Habits of the Glass-house Tomato-Moth, *Hadena oleracea*. *Annals of Applied Biology*, VII, No. 1.

LOWE (1900). Note on *T. pronubana*. *Ent. Rec.* XII, 316–7.

MILLIÈRE, P. (1859). *Iconographie et description de chenilles et Lépidoptères*, Nouvelle Série, Tome I, 382.

MOLINAS (1914). Sur un parasite des œuillets cultivés. *Jl. Agri. pratique*, Paris, XIX, 662–3.

PACKARD, A. S. (1898). *Text Book of Entomology*, 183.

POULTON, E. B. (1890). External Morphology of the Lepidopterous Pupa. *Trans. Linn. Soc. Zool.* (2), V, 245–63.

—— (1892 and 1903). Colour Relations between certain Lepidopterous Larvae and their Surroundings. *Trans. Ent. Soc. Lond.*

SASSCER, E. R. and BORDEN (1917). Fumigation of Ornamental Green-house Plants with Hydrocyanic Acid Gas. *U.S. Dept. Agri. Bull.* 513.

SICH, A. (1915–6). Life-cycle of *T. viridana*, L. *Proc. S. Lond. Ent. Soc.*

TRÄGÅRDH, I. (1917). Investigations into the Insects injurious to Spruce and Pine cones. *Meddelanden från Statens Skogsförsoksanstalt*, Heft 13–4, 1153.

EXPLANATION OF PLATE XV.

Carnation plants, showing injury resulting from attack by *T. pronubana*.

Plant A. Healthy unattacked plant.
B. 4–5 weeks after hatching of eggs on plant.
C. 6–8 weeks after emergence of young larvae; plant dying.

(*Received March* 18*th*, 1924.)

CERTAIN ASPECTS OF THE DAMAGE TO OATS BY THE FRIT FLY

By J. C. F. FRYER and J. E. COLLIN.

(With 3 Text-figures.)

Foreword. Since 1918 research into various problems connected with Frit Fly (*Oscinella frit* L.) has been in progress at the Ministry's Pathological Laboratory, the investigations having in some instances been made in collaboration with workers at other institutions. As the chief function of the Ministry's laboratory is not research, the work has been carried on in an intermittent manner and has resulted in the accumulation of much unpublished material which—if time had permitted—would have formed the basis for several progress reports instead of having been used merely as a stepping-stone from one experiment to another. Many of these earlier experiments have now served their purpose and are already out of date, but the following study, which deals in the main with the attack of the second brood of the Frit Fly on the oat grain, may still prove of interest and also serve as an introduction to other work carried out more recently.

The opportunity may be taken of expressing a debt of gratitude to all those who have assisted us directly or indirectly—notably to Mr Roebuck of the Midland Agricultural College, to Mr Robson of the East Anglian Agricultural Institute, to Mr Cecil King of Long Sutton, and the Lord Wandsworth Trustees who kindly gave facilities for the experiments to be carried out on their estate.

A. *The Damage to the Oat Kernel.* In 1918 plots were sown at Long Sutton, Hants., with "White Abundance" and "Black Tartarian" oats on March 18th and April 25th, one plot of each variety and of each sowing being left as control, a second plot receiving 1 cwt. per acre of sulphate of ammonia at the time of sowing, and a third being top-dressed with nitrate of soda on June 9th when the Frit attack on the young plant was causing obvious signs of distress in the crop. So far as this attack on the young plant is concerned, it may at once be stated that the value of early sowing was conclusively shown, but no differences justifying any deductions arose out of the manurial treatments or their absence. At harvest, large samples were gathered from different points scattered over each plot and the resulting grain was then spread out on a flat surface to a depth of one kernel only. The grain-covered area obtained in this way was again sampled, every grain being taken from equal-sized "plots" in different parts of the whole area. This second sample, consisting of 400–1000 grains, was examined by opening each grain in order to discover which were sound, which had been damaged by Frit and which were "blind," the results being as follows:

White (Abundance)

Sowing	Plot	Grains examined	% sound	% frit	% blind
Early	Control	430	90	5·8	4·2
	S. of A.	641	90·5	5·6	3·9
	N. of S.	1211	89·7	5·1	5·2
	Average all plots	2282	90	5·4	4·6
Late	Control	497	74·3	16·1	9·6
	S. of A.	937	73·3	19·3	7·4
	N. of S.	1029	70·7	17·2	12·1
	Average all plots	2463	72·4	17·8	9·8

Black (Tartarian)

Sowing	Plot	Grains examined	% sound	% frit	% blind
Early	Control	1342	92·1	3·2	4·7
	S. of A.	1095	89·1	4·1	6·8
	N. of S.	780	89·5	5·7	4·8
	Average all plots	3217	90·5	4·1	5·4
Late	Control	1070	76·5	14·9	8·6
	S. of A.	957	73·5	14·8	11·7
	N. of S.	852	66	21	13
	Average all plots	2879	72·4	16·6	11

The loss in grain which is undoubtedly due to Frit thus works out at 4 per cent. at least in the early-sown and 16 per cent. in the late-sown, while there were in addition a certain number of "blind" or infertile spikelets, that is to say, spikelets in which the grain had never developed, though outwardly they might appear practically normal. The cause of this blindness is unknown; it may in some cases be due to Frit but it can seldom be attributed to this pest with any certainty and blind grains are therefore counted separately in all the subsequent tables. Evidently late sowing renders the crop more susceptible to the second brood of Frit while it appears to influence, though to a less extent, the percentage of blind spikelets.

The considerable loss in sound grain indicated by this experiment, suggested that the loss to the country due to the second brood attack of the fly had not been sufficiently realised and arrangements were made to obtain samples at harvest from oat crops throughout England and Wales. As a result, in 1918 183 samples were received from 30 different counties, and in 1919 161 samples from 39 counties. The grain was dealt

Oat Trials, 1918 *and* 1919. *Sampling of Grain for Frit Attack.*

WHITE OATS

	1918 (%)				1919 (%)			
County	Cases	Sound	Frit	Blind	Cases	Sound	Frit	Blind
Northumberland	—	—	—	—	6	93·1	0·4	6·5
Durham... ...	2	87·5	3·3	9·0	—	—	—	—
Cumberland ...	—	—	—	—	4	95·7	0·6	3·7
Yorks.	4	89·3	3·0	7·7	6	84·9	7·8	7·3
Lancs.	11	86·0	7·5	6·5	7	83·85	9·45	6·7
Cheshire ...	4	87·8	5·7	6·5	4	83·4	10·9	5·7
Lincs.	3	78·5	15·6	5·9	—	—	—	—
Notts.	1	92·7	4·6	2·7	4	45·2	38·5	16·3
Derby	8	89·85	5·3	4·85	1	83·0	13·5	3·5
Leics.	—	—	—	—	1	62·5	9·5	28·0
Staffs.	1	91·6	2·7	5·7	3	73·9	22·8	3·3
Salop	11	65·1	24·8	10·1	4	69·4	20·0	10·6
Warwicks. ...	6	83·8	11·1	5·1	1	43·2	30·0	26·8
Norfolk	3	83·2	12·6	4·2	1	84·6	7·5	7·9
Cambs.	5	87·1	9·15	3·75	1	65·4	24·3	10·3
Northants. ...	1	73·5	21·6	4·9	10	66·3	22·8	10·9
Suffolk	2	92·1	3·95	3·95	1	76·7	9·4	13·9
Essex	—	—	—	—	2	86·05	9·7	4·25
Beds.	6	77·2	18·0	4·8	3	71·9	16·3	11·8
Kent	1	93·5	3·9	2·6	3	69·76	23·26	6·96
Bucks.	—	—	—	—	1	66·3	29·8	3·9
Oxon.	3	86·8	9·0	4·2	3	68·7	16·7	14·6
Middlesex ...	—	—	—	—	1	79·1	15·2	5·7
Berks.	1	91·8	5·5	2·7	3	78·4	15·3	6·3
Hants. & I. of W.	9	85·4	8·5	6·1	3	64·4	18·6	17·0
Dorset	3	93·8	2·4	3·8	—	—	—	—
Worcs.	7	80·4	11·7	7·9	19	46·75	44·95	8·3
Hereford ...	—	—	—	—	1	65·7	27·3	7·0
Gloucester ...	5	87·0	10·25	2·75	—	—	—	—
Wilts.	3	91·3	3·8	4·9	—	—	—	—
Somerset ...	1	95·0	2·8	2·2	2	80·55	12·95	6·5
Devon	5	85·95	7·9	6·15	6	83·6	12·6	3·8
Cornwall ...	5	80·7	13·5	5·8	2	83·5	10·15	6·35
Anglesey ...	1	96·7	1·1	2·2	2	87·6	0·6	11·8
Denbigh ...	5	83·6	7·6	8·8	2	85·25	11·55	3·2
Flint	2	85·85	3·9	10·25	2	75·35	20·6	4·05
Carnarvon ...	3	91·63	2·93	5·43	7	84·8	6·3	8·9
Merioneth ...	3	89·86	1·76	8·36	—	—	—	—
Radnor	—	—	—	—	2	63·45	30·0	6·55
Cardigan ...	—	—	—	—	4	86·1	0	13·9
Brecon	1	81·5	10·6	7·9	—	—	—	—
Carmarthen ...	—	—	—	—	2	93·05	2·7	4·25
Glamorgan ...	1	83·3	7·5	9·2	—	—	—	—
Total for England and Wales	127	84·1	9·7	6·2	124	72·9	18·5	8·6

Oat Trials, 1918 *and* 1919. *Sampling of Grain for Frit Attack.*

BLACK OATS

	1918 (%)				1919 (%)			
County	Cases	Sound	Frit	Blind	Cases	Sound	Frit	Blind
Northumberland	—	—	—	—	2	96·45	—	3·55
Durham... ...	2	94·45	0·45	5·1	—	—	—	—
Yorks.	1	76·6	16·9	6·5	—	—	—	—
Lancs.	2	81·05	11·65	7·3	—	—	—	—
Cheshire ...	1	61·7	12·3	26·0	1	90·8	1·6	7·6
Lincs.	2	81·0	9·75	9·25	—	—	—	—
Derby	2	92·4	4·45	3·15	—	—	—	—
Warwicks. ...	2	55·65	36·8	7·55	2	63·6	30·2	6·2
Cambs.	2	82·95	11·55	5·5	2	73·65	12·0	14·35
Northants. ...	3	74·1	17·8	8·1	—	—	—	—
Suffolk	—	—	—	—	2	78·5	13·05	8·45
Herts.	—	—	—	—	1	91·0	2·8	6·2
Beds.	2	85·8	9·55	4·65	—	—	—	—
Kent	2	81·2	11·85	6·95	1	65·2	28·0	6·8
Surrey	1	92·3	4·2	3·5	—	—	—	—
Sussex	4	91·2	4·4	4·4	—	—	—	
Bucks.	1	85·6	8·2	6·2	—	—	—	—
Oxon.	2	91·8	5·0	3·2	3	62·4	28·0	9·6
Berks.	2	81·05	11·05	7·9	1	76·8	7·0	16·2
Hants. & I. of W.	10	90·0	4·1	5·9	—	—	—	—
Dorset	8	89·4	3·1	7·5	—	—	—	—
Worcs.	2	79·3	12·35	8·35	4	49·9	40·3	9·8
Gloucester ...	2	82·1	10·2	7·7	1	81·3	15·4	3·3
Wilts.	5	82·9	8·8	8·3	—	—	—	—
Somerset ...	3	82·6	8·0	9·4	3	70·0	16·4	13·6
Devon	7	81·2	8·1	10·7	6	77·6	16·8	5·6
Cornwall ...	12	86·55	5·55	7·9	2	89·1	0·25	10·65
Anglesey ...	2	91·1	0·5	8·4	1	92·4	3·7	3·9
Carnarvon ...	2	91·7	1·7	6·6	1	79·2	4·7	16·1
Radnor	—	—	—	—	2	90·6	4·85	4·55
Brecon	2	85·95	7·35	6·7	—	—	—	—
Monmouth ...	2	94·75	2·55	2·7	—	—	—	—
Glamorgan ...	1	93·9	0·2	5·9	2	71·25	24·75	4·0
Total for England and Wales ...	89	85·2	7·5	7·3	37	75·2	16·6	8·2

with in the manner indicated previously and the details so obtained are given in tables dealing respectively with White and Black oats (precise varieties mostly unknown).

It will be seen that the number of samples received from any one county or district was hardly sufficient to justify any numerical generalisation on a basis either of counties or districts, but it is evident that the material loss caused by the second brood attack is very great. Even in 1918, a season in which Frit Fly was less prevalent than usual, the actual loss in grain is heavy. One hundred and twenty-seven samples of white oats gave an average of 9·7 per cent. grain per sample damaged by Frit. In 1919—the following year—the attack was patchy, distinctly bad in certain counties but relatively light in others: on the whole, perhaps slightly worse than the average. In that year 124 samples of white oats prove to have no less than 18·5 per cent. of grains per sample damaged by Frit, while in case it may be considered that this result is due to the receipt of an excessive number of samples from the areas most attacked, it may be mentioned that on these figures the county average works out at 15·8 per cent. per county. In each year, black oats appear slightly but definitely better than white oats, as in the case of the Long Sutton experiment, but in view of the wide range shown by the figures (black oats in 1918 ranged from 0–36·8 per cent. and in 1919 from 0–40 per cent.) it would not be safe to assert on these figures alone that the difference is significant.

Fig. 1.

The information gained in 1918 caused greater attention to be paid in 1919 to the grain attack, and in addition to obtaining the statistics given in the previous tables, arrangements were made to carry out experiments at three centres to attempt to discover something further as to the factors influencing the attack.

Previous experiments seemed to indicate that there was a critical period in the blossoming or early stage of the oat-panicle when the plant was most susceptible, and also a period of greatest prevalence of the brood of Frit Flies responsible for the attack, with the result that maximum infestation occurred when the two coincided. It was therefore decided to screen plants at intervals during the development of the seed-head and to release periodically others that had been screened prior to the emergence of the panicle and so attempt to prove or disprove the above supposition. The screening was done by means of tiffany bags supported by stakes (Fig. 1), and each set of plants screened or released was labelled with the date when screened or exposed, the panicles being carefully collected at harvest and every grain examined for Frit infestation. This method needed excessive care in the fixing of the bags, and proved troublesome, the attention of mice, rats, and dogs being attracted to the bags and of children to the useful sticks by which they were supported. In consequence the results obtained at two centres are too irregular to quote in detail, but the Long Sutton experiments were more fortunate, although even here a young retriever rendered certain of the baggings abortive, while in one or two cases rats or mice nibbled holes in the tiffany and may have allowed Frit Flies to enter. Fortunately few of the accidents occurred in connection with the control plants, that is to say, those covered from a date prior to the emergence of the ear from the sheath until harvest, and Frit Fly injury was absent from these ears covered throughout the experiment except in the case of one bag on Plot II which had been disturbed by the dog. It is therefore clear that the bags were applied at a sufficiently early date. Further, the oats within the bags developed normally without differing appreciably from the normal uncovered plants.

The details of the experiment were as follows:

Plots, not measured but exceeding 1 acre, were sown:

Plot I.	March 28th,
Plot II.	April 17th,
Plot III.	„ 24th,
Plot IV.	May 15th,

the irregularity in date being due to weather conditions and cultural difficulties. Later work in connection with the "bagging" was carried out as follows:

June 18*th*. Plot I. Attached 15 bags.
Plot II. „ 3 „
Plot III. „ 15 „
Plot IV. „ 3 „

On this date the top spikelet of the panicle was just visible within the leaf sheath on one or two stems on Plot I, but such stems were not bagged. No panicles were visible on the other plots and the plants on Plot IV were only about 6 inches high and difficult to enclose. On this date also the plots were sampled as follows in order to obtain some measure of the first brood attack. Ten stations, approximately equidistant, were selected in each plot and at each station one foot of row was chosen at random (so as to avoid any unconscious selection of good or bad plants) and the oats from the measured length dug up and removed to the laboratory for examination. The average number of plants per foot run of row was approximately 15 (extremes = 14·7 and 16·9) and the results as follows:

	Percentage of sound plants	Percentage of plants attacked but sending up stems bearing panicles	Percentage seriously attacked, not sending up stems bearing panicles
Plot I	77·7	16·9	5·4
„ II	89·0	4·9	6·1
„ III	87·6	0·6	11·8
„ IV	54·4	12·9	32·7

It is difficult to suggest what reliance can be placed upon these figures as the experimental error may be very large; on the other hand, any serious attempt to ensure accuracy would have required a large staff of investigators, while the consequent interference with the crop might not improbably have prevented the main experiment. Accepting the figures as they stand, column 3 coincides with expectation and with the appearance of the crops in the plots. Column 2 lends some support to the theory that it is possible to sow the oat crop too early, for Plot II, which gives the best results (in at least so far as the number of young plants not attacked by flies of the first brood is concerned), also, as will be seen subsequently, came out well in the grain attack. It is not proposed, however, on account of the difficulty in estimating first brood attack, to base detailed deductions on these figures and they are inserted mainly in order to preserve the records complete.

July 1*st*. Plot I. Attached 4 bags. Released 4.
Plot II „ 3 „
Plot III. „ 4 „ „ 4.
Plot IV. „ 3 „

On this date the panicles were clear of the sheath on Plots I, II and III, and were breaking out on Plot IV. The following notes were made as to the conditions in detail:

Plot I. [1]Upper spikelet, 1st and 2nd grains fertilised.
Middle spikelet, 1st grain fertilised, 2nd grain pollen just shed.
Lower spikelet, 1st grain not fertilised.
Plot II. Upper spikelet, 1st grain fertilised, 2nd grain pollen just shed.
Middle spikelet, 1st grain pollen just shed, 2nd grain not fertilised.
Lower spikelet, 1st grain quite immature.
Plot III. Upper spikelet, 1st grain fertilised, 2nd grain pollen just shed.
Middle spikelet, 1st grain not fertilised.
Lower spikelet, 1st grain quite immature.
Plot IV. Upper spikelet, 1st grain pollen just ready to shed, 2nd grain immature, and remainder very immature.

On *July 11th and July 25th* the same procedure was followed both as regards numbers of bags put on and bags taken off in the case of each plot. On July 11th the following notes were made as to the conditions of the kernel:

Plot I.	Upper spikelet Middle ,,	Grains milky and full size.
	Lower ,,	Grains half size.
Plot II.	Upper spikelet	Larger grains milky.
	Middle ,,	Grains hardly full size.
	Lower ,,	Grains quite small.
Plot III.	Upper spikelet	Larger grains becoming milky.
	Middle ,,	Grains small (under half size).
	Lower ,,	Grains quite small.
Plot IV.	Upper spikelet	Grains half size.
	Middle ,,	Grains quite small.
	Lower ,,	Grains not fertilised.

By July 25th the plots were turning colour, Plots I and II being nearly ripe, and no easy method of differentiating between the condition of the grain in the different plots was found. On August 6th the

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[1] The terms "upper," "middle," and "lower" refer to the position of the spikelet in the panicle, the "upper" being the terminal spikelet, the "lower" a spikelet borne upon a lowermost branch of the panicle, and the "middle" a spikelet as near as possible halfway between upper and lower. The 1st grain refers to the larger of the two grains usually found between each pair of glumes, the 2nd grain being the smaller one arising from near the base of the first.

"bagged" and "released" plants were judged ripe and were removed to the laboratory for detailed examination, which gave the following results:

	Plot I			Plot II		
Date	No. of bags	Total no. of grains	% attacked by frit	No. of bags	Total no. of grains	% attacked by frit
Bagged						
Throughout (control)	3	820	0	2*	429	0·2
July 1	4	1208	1·9	3	784	1·5
„ 11	4	1625	2·7	3	934	2·5
„ 25	4	1897	2·2	3	1254	2·3
Released						
July 1	4	807	0·8	—	—	—
„ 11	4	1065	0	—	—	—
„ 25	4	888	0	—	—	—

	Plot III			Plot IV		
Date	No. of bags	Total no. of grains	% attacked by frit	No. of bags	Total no. of grains	% attacked by frit
Bagged						
Throughout (control)	3	466	0	3	382	0
July 1	3*	532	1·1	3	638	0·15
„ 11	4	894	2·9	3	798	3·3
„ 25	4	936	2·4	3	1115	9·7
Released						
July 1	3*	647	2·5	—	—	—
„ 11	4	528	0·3	—	—	—
„ 25	4	510	0	—	—	—

* One bag in each of these cases was destroyed by a dog and is not included.

In the case of the control in Plot II it may be mentioned that the dog completely destroyed one bag and disturbed another sufficiently to account for the 0·2 per cent. infestation. For convenience in discussion the above figures have been reduced to the form of a chart and with Mr Cunliffe's permission a curve showing the prevalence of Frit Flies at Oxford between July 7th and August 6th, 1919 has been added(1).

Certain points are shown very clearly by the graph (Fig. 2):

(I) *Two factors were operating throughout the experiment, the first being a variation in the state of susceptibility of the oat to an attack, and the second a variation in the number of flies present on the plots.*

This was to be expected but the definite demonstration was not without value and special interest attaches to the proof that the appearance of the second brood of the fly was well defined and was not swamped by the overlapping of the two broods. It will be noticed that the maximum infestation on Plots I, II and III does not rise above 29 per 1000 and no increase of infestation took place after July 11th, while on Plot IV the figure of 97 per 1000 was recorded on July 25th. It is therefore practically certain that the oats on Plots I, II and III had passed the stage of greatest susceptibility before the great increase in the number of flies (due to the emergence of the second brood) began about July 11th. On the other hand, the fact that the curves showing the increase in infestation on Plot IV and the increase of flies at Oxford are more or less parallel, equally suggests that in this case the period of maximum susceptibility of the plant coincided with the maximum prevalence of the flies.

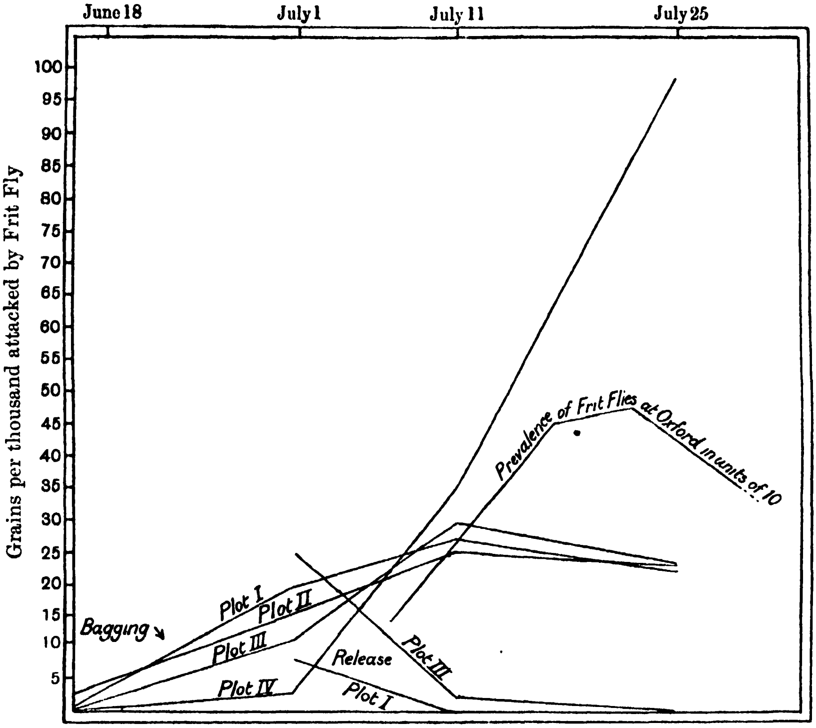

Fig. 2.

The deduction as a whole is satisfactory as it indicates the possibility of "dodging" the grain infestation by appropriate timing of the crop exactly as is being attempted in the case of the first-brood attack on the young plant, earliness in each case being the great desideratum.

(II) *The period of maximum susceptibility of the oat spikelet lies approximately at the time of fertilisation.*

As mentioned above, by July 11th, Plots I, II and III had reached their maximum infestation and were practically no longer susceptible to infestation for the attack of 3 per 1000 obtained in the oats released on Plot III on this date may well be due to a small retarding influence on the plants owing to their having been bagged since June 18th: it is in any case too slight to invalidate the deduction. On the other hand, Plot IV on July 1st was not appreciably infested although the other plots prove that sufficient flies were available to cause an infestation up to 19 per 1000. It may be concluded therefore that the oats were most attractive to the flies for the purpose of oviposition between the stages represented by the oats on Plot IV on July 1st and the oats on Plot III on July 11th.

On July 1st the most forward grain in the panicles on Plot IV showed pollen just ready to be shed, while on July 11th the most backward grains on Plot III, though fertilised, were still quite small—at any rate under half their ultimate size. From this it might be deduced that the condition of maximum susceptibility (to oviposition) in the oat lies between fertilisation and the time when the oat kernel is half-size, a period which, in these experiments, cannot have lasted for much more than a week. It should, however, be noted that up to July 1st the oats on Plot I had been more heavily infested than those on Plot III, while immediately after this date (as proved by the released bags) the position was reversed. From this it would appear that the maximum susceptibility to attack of the oats on Plot I occurred just before July 1st and for those on Plot III just after. In view of the state of the oat panicles about this date, the above consideration would appear to limit still more closely the stage of maximum susceptibility to the time of fertilisation (for the spreading of the time of infestation over several days might be largely accounted for by the fact that oats in a panicle do not all arrive at a state for fertilisation at the same time). The period of susceptibility is thus surprisingly short and perhaps can hardly be accepted as a result of one year's experiments only. Its commencement is indicated by Plot IV only and is based on an examination of 638 grains, and it is possible that a larger experiment might indicate a degree of susceptibility prior to fertilisation. In this connection reference should be made to Roebuck's observations (2) on the occurrence of Frit larvae feeding in the oat panicle prior to its emergence from its sheath—which would appear to be entirely inconsistent with the results obtained in the present experiment. However, it would seem clear that an explanation of Roebuck's observations must be sought along lines other than by advancing to an earlier stage the beginning of the true period of grain susceptibility. At Long Sutton, where oat plants were protected from Frit Fly throughout, 11 bags containing 1911 grains had only one grain attacked by *Frit* and even this one is explicable on the grounds of experimental error (the dog) rather than by assumption of infection prior to "bagging." That absence of flies was not responsible for this result is proved by the fact that Plots I, II and III became infested as soon as flowering occurred—Plot IV in a less advanced stage being hardly attacked. It is not therefore considered that Roebuck's results necessitate any great advancement of the stage of susceptibility of the oat grain prior to flowering of the oat. It may be asked whether Roebuck's results are not explicable by assuming that infestation took place at the close of the first attack on the young plant, which in his case may have been prolonged to a later date than usual, and to have included the stage when the plant was about to throw up the flowering stem. The larvae would then have been carried up with the "ear" with the result he has recorded. The parents of these aberrant larvae may, as he suggests, have been second brood flies emerging earlier than usual or late first brood flies, but so far as the oat is concerned, this appears to be immaterial.

Turning now to the end of the stage of susceptibility of the oat grain, the evidence available is considerable. Plots I, II and III on July 11th comprised grains varying from "half-sized" to "small" but none were in the recently fertilised condition. By the bagging experiments no increase in the infestation appears to have occurred after July 11th and this result is fully confirmed in the case of Plot I by the release experiments—panicles released on that date escaping attack altogether. The panicles of Plot III, also released on July 11th, comprising 528 grains proved attacked only to the extent of 3 per 1000—an attack which in any case is sufficiently low to be regarded as substantially in agreement with the bagging experiments. The slight difference is, however, explicable on the assumption that the bags which had been on since June 18th had slightly delayed the progress of ripening and so held a few grains in a susceptible condition for a day or two longer than the average for the plot. It is clear that if Plots I, II and III had been to any extent susceptible they must have been attacked, since Plot IV after July 11th showed an increase of 64 damaged grains per 1000 between this date and July 25th, proving the presence of ample flies. It would therefore seem clear that the oat grains on these experimental plots were at the latest no longer susceptible by the time they had

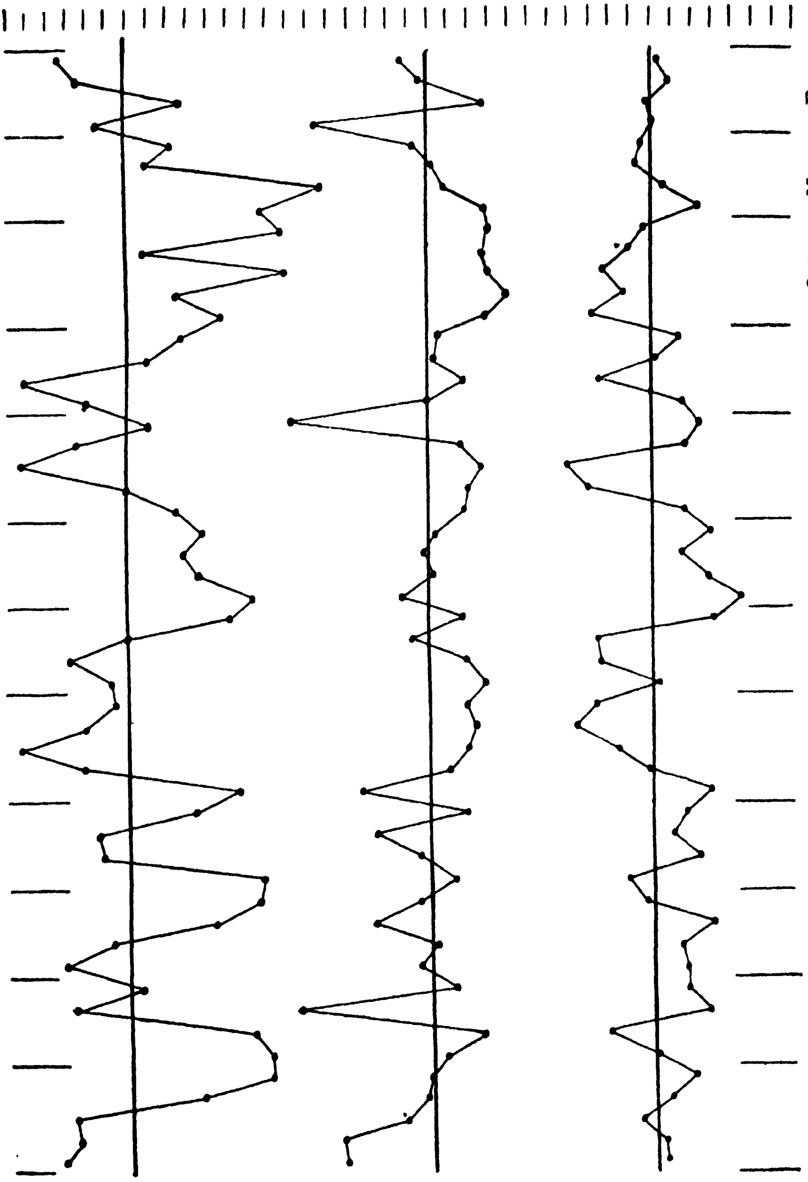
Dec.
Nov.
Oct.
Sept.
Aug.
July.
June.
May.
April.
Mar.
Feb.
Jan.
Temperature
SCALE :—(Degrees Fahrenheit).
Rainfall
(5 Millimetres).
Sunshine
(Hours).

Fig. 3.

swelled to half their full size and there is at present no adequate reason for not extending this deduction to oats of the Abundance varieties in general, if not to all varieties. As the maturation of the grain is influenced by weather conditions, it may be enquired as to whether July, 1919 conduced to abnormally rapid ripening. This, however, can hardly have been the case as the month was cooler than the average, with less sunshine and about a normal fall of rain as is shown in the accompanying weather chart[1] for S.E. England, where the variation above and below the average mean is given for temperature, rainfall and sunshine. Finally, before leaving this experiment, one other possible disturbing factor may receive mention. On the ordinary oat field the plants are all of the same age and approximately in the same condition throughout their growth, while in the experiment four large plots in different stages were present. In the latter case therefore the flies were—in theory at all events—given the choice of oats in all conditions and so might have been able to select only those in the most suitable state for oviposition, neglecting others which, though capable of sustaining the larvae, were nevertheless less attractive. This possibility cannot be entirely disposed of, since the oat when in flower, for instance, may emit some very attractive scent which would cause flies to desert plots not in flower. The experimenters do not consider, however, that this factor of choice was operative to any extent and the final view is expressed that the normal period of susceptibility in the oat grain begins very shortly before fertilisation and closes before the grain reaches half its ultimate size, with the probability that maximum susceptibility occurs at the time of fertilisation.

B. *Variety Trials*. Concurrently with the experiments just recorded, a series of variety trials was carried out which may be discussed somewhat briefly. Observations and experiments carried out in 1918 and previous years indicated that if several varieties of oat were sown on neighbouring plots on the same date, great variation could be detected in the extent to which the several kinds suffered from the attack of Frit. As the discovery of a resistant oat appeared to be one of the most likely means of solving the principal problem, considerable attention was given to the subject, but progress was found to be slow. Varieties which in one locality and one season gave promising results appeared to fail in another locality and another season, while, further, there was found to be a very real difficulty in estimating scientifically the infestation by

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[1] **Reproduced from Miscellaneous Publication No. 33 of the Ministry of Agriculture and Fisheries.**

Frit Fly of the young plant. Varieties of oats have different habits of growth and react differently when attacked by the fly—some for instance appear very susceptible but recover well, while others are less susceptible but have little power of recovery. In the case of a variety trial, therefore, it is difficult to obtain any standard of comparison which is reliable and it was intended to hold over the following results until the precise reaction of oat varieties to the fly had been worked out in detail. As, however, other investigators are publishing results of variety trials, it seems well to follow suit, with the warning however that in the opinion of the investigators, the method of conducting the trials was not such as to give results of real accuracy.

The experiment was as follows: 13 varieties were sown on April 25th by means of a hand drill in narrow strips running the entire length of the field. On June 18th, the plots were sampled according to the method described on p. 454 except that owing to the plots being narrow but half the number of plants were taken. These sample plants were examined in the laboratory and were classified into: plants not attacked; plants attacked but sending up a panicle; plants attacked and showing no signs of "earing" and therefore more damaged than those in the previous category. The result was as follows:

	Plants not attacked	Attacked, but sending ear	Attacked, not sending ear	Relative numbers of plants in sample
1. Thousand Dollar	95·6	—	4·4	93
2. Supreme	91·3	2·1	6·6	94
3. Victory	83·3	5·1	11·6	62
4. Record	86·8	—	13·2	70
5. Yielder	77·4	2·6	20·0	87
6. Banner	75·3	1·0	23·7	100
7. Golden Rain	63·9	7·4	28·7	97
8. Black Tartarian	66·7	3·3	30·0	62
9. Abundance	56·6	10·9	32·5	86
10. Sandy	50·0	10·6	39·4	79
11. Leader	43·7	6·3	50·0	50
12. White Tartarian	39·4	10·6	50·0	68
13. Potato	6·1	7·7	86·2	67

In this list the varieties are arranged according to the third column—Thousand Dollar, which showed the smallest percentage of plants seriously attacked, being placed first. An alternative order of merit can be obtained if column 1—percentage of plants not attacked—is taken, but it will be seen that very little alteration in the order is caused—Record changing places with Victory and Black Tartarian with Golden

Rain. No further comment is needed except to point out that the numbers represent only the plants remaining on June 18th, no account being taken of plants which from Frit attack or other reasons had died out and disappeared previously. This dying out of plants is a difficult factor to deal with in a field experiment but it is one to which further attention should be given. So far as our observations go there is in practically every case a progressive loss of plants during the first six weeks after the crop appears; it may be due to frit, to wireworm, or other pests, and even perhaps to inclement weather conditions. The essential fact, however, is that the loss is not uniform in regard to a series of varieties and where the results of an experiment are based upon yield, entirely erroneous conclusions may be drawn. In order to indicate how considerable may be the effect, a fourth column on the preceding table indicates the relative numbers of plants in the samples—the variety with the highest number being rated at 100. Of course, neither the sowing nor sampling was sufficiently uniform to justify the expectation of more than approximately the same number of plants in each sample, but the difference is so great that it cannot be due to experimental errors of this description.

After sampling on June 18th, the variety plots were left until harvest when they were again sampled and the spikelets analysed for the second brood attack, three categories being recognised: sound grain, grain definitely attacked by Frit, and blind spikelets. The results were as follows:

Variety	% frit	% blind	% sound	Order in Spring attack	1922 % frit
1. Golden Rain	7·7	5·3	87·0	7	—
2. Victory	9·3	14·0	76·7	3	16·5
3. Yielder	9·3	11·8	78·9	5	17·6
4. Black Tartarian	9·5	6·4	84·1	8	21·6
5. Thousand Dollar	10·2	2·2	87·6	1	—
6. Supreme	10·3	13·7	76·0	2	20·7
7. Banner	11·0	8·0	81·0	6	—
8. Record	14·4	16·3	69·3	4	—
9. Leader	16·7	12·2	71·1	11	22·4
10. Sandy	17·1	11·5	71·4	10	—
11. Abundance	18·2	10·9	70·9	9	24·0
12. Potato	21·2	17·6	61·2	13	15·5
13. White Tartarian	21·7	12·8	65·5	12	—

In column 4 of the above table is given the position of the variety in regard to the spring attack as indicated in the table on p. 462. It is evident that, judged by the percentage of frit-destroyed grains, there is extraordinarily little correlation between the respective resistance to the spring attack and to the grain attack, suggesting that early maturity does not necessarily follow upon rapid growth in the plants' earlier stages. Also, the percentage of sterile spikelets appears to have no correlation with either of the Frit attacks and on this particular occasion it is difficult to believe that this pest was in any way concerned in the production of blindness.

Finally, reference may be made to a fifth column in the table which gives the percentage of grains attacked by Frit in a small variety trial carried out at Harpenden in 1922. All the varieties tested at Long Sutton were not grown at Harpenden, but it is of interest to note that, with one exception, where the same varieties were grown, each retains the same position in the list. For the behaviour of Potato, the most attacked variety at Long Sutton and the least at Harpenden, no explanation can be offered. At this point this series of trials may be left as the results are not considered to be sufficiently accurate to justify any elaborate deductions. The difficulty in obtaining in variety trials such as those described any results of a known order of accuracy appeared so great that it was decided to abandon field trials and to attempt on a small scale to discover the fundamentals concerned in the reaction of the young oat plant to Frit attack. A succeeding paper will deal with such an attempt and also with certain experiments to determine the exact period of susceptibility of the oat plant to the spring attack.

REFERENCES.

(1) Cunliffe, N. C. Preliminary observations on the habits of *Oscinella frit*, Linn. *Annals of Applied Biology*, viii, 114.

(2) Roebuck, A. Frit Fly in relation to Blindness in Oats. *Ibid.* vii, 178.

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OSCINELLA FRIT, LINN. AN INVESTIGATION TO DETERMINE HOW FAR VARIETAL DIFFERENCES MAY INFLUENCE INFESTATION OF THE OAT PLANT

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I. INTRODUCTORY REMARKS.

ALTHOUGH it is realised that a number of different workers have been engaged on the problem of varietal resistance, little appears to have been published on this subject and the only papers which have been consulted are those by Harper Gray (2), Fryer (1), Kleine (3) and Petherbridge (4). Kleine's records fail to distinguish adequately between the large number of varieties used, while both Harper Gray and Fryer base their conclusions on the percentage of damaged *plants*, or some variation of this method of evaluation. As yield is dependent on the number of stems capable of producing panicles as well as on the extent of the grain infestation, this method of differentiating between varieties is not effective unless the infestation varies between very wide limits. Taking an extreme case, two varieties may show 30 per cent. and 60 per cent. unattacked plants respectively. If the plants in each variety are capable of producing four stems, the unattacked stems in the first case will range from 30–82·5 per cent. and in the second case from 60–90 per cent. Thus there may be the same total production of effective stems although the unattacked plants differ by 30 per cent. in the two cases. No reliable data are available to enable a comparison to be made between the many different varieties of oat in relation to infestation by *O. frit.* A series of investigations, of which the following study is a member, has been planned to determine (1) whether varietal differences influence infestation under similar environmental conditions, (2) how varieties react to varying environmental conditions, and (3) the factors controlling the variations in intensity of infestation, particularly when the limits of the variation are sufficiently wide to be of practical importance.

It is not claimed that the results recorded here are in any way final, because other variations in environment may react on these varieties of the oat quite differently, producing slight physico-chemical or physiological changes which even though only slight may be sufficient to alter susceptibility very materially, probably through the operation of a time factor. However this may be, it is suggested that only by means of detailed and statistical analyses can reliable conservative conclusions be drawn from the results of experiments of this nature. Such trials whether designed as frit fly investigations or for more general purposes usually show different results on the part of the varieties concerned. It is then often forgotten that although the facts observed may be true under the conditions of the experiment yet no generalisation is permissible unless the experimental errors can be defined, for frequently these errors are so large that observed differences, apparently considerable in themselves, may not be significant.

Finally, it should be recorded that the statistical analyses were made at Oxford, while Mr Gibson (now of the Scilly Islands Experimental Station) carried out most of the practical work at Harpenden.

Our sincere thanks are due to Mr R. A. Fisher, Statistician, of Rothamsted, who has kindly looked through this paper in MSS. and made valuable suggestions.

II. METHOD OF PROCEDURE.

From previous experience it was concluded that adequate analyses could be made on small plots only, the stem analysis having to be conducted in the shortest possible time. Even on plots of the small size used during this investigation it was noted that environmental conditions were not absolutely uniform. To acquire preliminary data as to the effect of the environmental factors, the investigation was conducted in duplicate in widely separate localities, one set of experiments being arranged in the garden of the Pathological Laboratory at Harpenden, Hertfordshire, and the other on the University Farm, Sandford-on-Thames, Oxon., the experiments being identical except for locality. On plots running north and south and measuring 27 feet in length, fourteen drills 1½″ deep were made at distances of 9 inches. In these drills the twelve varieties recorded below were sown on 18. iv. 23, as evenly as possible, at the rate of 1¼ ounces of seed per 27 feet (which was calculated to be a mean field rate), the varietal order, which was the same in each series, being *Supreme, Victory, Record, Leader, Black Tartarian, Yielder, Goldfinder, Captain, Sir D. Haig, Superb, New Abundance* and *Scotch Potato*. The outside drills (*Abundance*) merely maintained normal conditions for the adjacent rows. Wire netting served to prevent rabbit and bird damage.

Our thanks are due to Messrs Garton, Ltd., Warrington, for their courtesy in supplying the necessary seed.

The following prearranged scheme of observations was followed where practicable during the growing season:

(1) the plants in the drills were counted on 18. v. 23;

(2) samples of each variety were pressed weekly for reference, each sample consisting of four plants, two of the mean type and one each of the mean extreme types;

(3) the rate of panicle shooting was observed;

(4) the dates of flowering (date pollen was ready to shed on the upper spikelets) were recorded;

(5) the dates when the seeds of the upper spikelets reached the "milky" stage were recorded;

(6) sampling for estimation of extent of infestation was conducted as follows:

(*a*) *Stem.* Strings were stretched across the drills at intervals of 4 feet and all the plants collected in that portion of each drill 4 inches on each side of the string. Thus for each variety about one-sixth of the total number of plants were available for analysis. The sample plants were carefully stripped and each stem, from the buds upwards, examined under the binocular microscope for evidence of frit infestation. Samples were collected on a prearranged date, 25. vi. 23, after which it was considered that newly-formed tillers would fail to produce panicles of importance in relation to yield[1]. It was found impossible in many cases to identify correctly the different stems of the plant and a rough classification of stems was adopted at Oxford, based on the length of the stem including the blade, the four classes used being buds, 1–6″, 6–12″, and 12″ and over. Stems below 1″ in length were usually covered by the sheaths and were included with the buds.

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[1] See p. 469.

(*b*) *Grain.* At Oxford the grain was sampled by collecting separately the panicles from the seven remaining sections when the seed had matured. For examination the panicles from each section were stripped, the spikelets well mixed and a number of spikelets from the centre of the small heap examined for infestation, the main and bosom grains being examined separately. At Harpenden the grain of each variety was collected in bulk. The panicles were stripped, the grain freed from the glumes, mixed, spread in layers one grain deep and sampled six times mechanically and regularly. In the case of the grain a certain difficulty presented itself, inasmuch as some ovules and seeds do not develop. Lack of development of the ovule may be due either to failure in the process of fertilisation or to the attack of the frit fly or other pest. Very immature seeds, as the result of infestation, may cease growth immediately after fertilisation, in which case the small fly larva may die or migrate leaving very little evidence of its work. As it was frequently impossible to determine exactly the cause of this "blindness" all doubtful cases have been included with the clean undeveloped grain under the heading of "blind" grain.

III. Experimental Data.

(*a*) *Stem.*

The minimum, mean and maximum numbers of plants per drill at Oxford on 18. v. 23 were 292, 446 and 598 respectively, the range being higher at Harpenden where conditions were better. The minimum, mean and maximum numbers of plants and stems in the samples taken on June 25 were (*a*) Oxford, 43, 63, 98 and 126, 172, 225, and (*b*) Harpenden, 78, 87, 156 and 307, 413, 741 respectively. Thus the plants at Harpenden produced 1·74 times the mean number of stems produced by the plants at Oxford. The mean infestations shown by the series of samples across the drills, the varieties being taken collectively, were (*a*) Oxford, 61·1, 56·4, 62·9, 56·4, 58·3, 56·4 per cent., and (*b*) Harpenden, 53·8, 62·3, 65·1, 67·9, 68·8 and 70·4 per cent. In the latter locality, the decreasing infestation was in the direction of the prevailing wind.

In Table I are shown the arithmetic mean percentage infestations of stems and grain for the twelve varieties of oats under observation at

Harpenden and Oxford, together with the standard errors of the means as determined from the formula $\sqrt{\frac{\Sigma d^2}{n(n-1)}}$, where d is the difference between the observed value and the arithmetic mean of the series, the number of observations (n) being small. The exposed stems only are included in these means, the small hidden tillers (buds) being omitted[1], and the records are set out in the plot order.

Table I.

Arithmetic mean percentage of infestation with standard error.

O = Oxford H = Harpenden

Name of variety		Stem (omitting buds)	Grain
Supreme	O	64·4 ± 2·9	42·4 ± 2·9
	H	71·1 ± 2·7	16·0 ± 0·7
Victory	O	58·0 ± 2·0	33·6 ± 1·8
	H	63·4 ± 2·4	17·0 ± 0·5
Record	O	58·2 ± 6·8	34·7 ± 2·7
	H	71·0 ± 2·4	13·9 ± 0·8
Leader	O	57·7 ± 4·9	37·8 ± 2·5
	H	66·9 ± 2·3	15·9 ± 1·6
Black Tartarian	O	65·8 ± 4·1	35·1 ± 1·8
	H	63·6 ± 2·6	20·7 ± 0·8
Yielder	O	65·2 ± 3·3	42·1 ± 3·3
	H	66·6 ± 3·3	16·0 ± 1·8
Goldfinder	O	46·1 ± 3·7	36·7 ± 1·5
	H	56·5 ± 6·7	15·6 ± 0·7
Captain	O	60·3 ± 3·8	38·1 ± 4·0
	H	66·0 ± 2·8	15·5 ± 0·6
Sir D. Haig	O	54·4 ± 6·0	32·2 ± 2·7
	H	63·7 ± 4·9	22·4 ± 1·3
Superb	O	68·3 ± 5·7	38·0 ± 1·7
	H	68·1 ± 2·8	13·7 ± 0·9
New Abundance	O	48·3 ± 6·0	34·9 ± 2·6
	H	58·0 ± 4·3	28·9 ± 1·6
Scotch Potato	O	56·0 ± 5·1	26·7 ± 2·4
	H	61·9 ± 3·7	13·4 ± 1·1

The sampling date for the stem infestation analysis was arranged originally for 7. vi. 23, but it was observed at Harpenden that eggs were not visible on the oat stems until the end of May. In consequence of this observation it was thought that the main attack had not occurred in that place and therefore the later date (25. vi. 23) was arranged. It was discovered afterwards that eggs were deposited below the surface of the soil. The sampling prearranged for 7. vi. 23, when it was possible to distinguish between main stems and tillers, having been conducted at Oxford, the results are recorded in Table II. In this table are shown the percentage infestations of main stems, exposed tillers, total stems omitting hidden tillers, together with the records of the later sampling for comparison. In each case the percentage was calculated from the samples treated as a whole.

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[1] See pp. 468 and 470.

Table II.

Percentage infestation of stem, Oxford.

Variety	Main stems	Exposed tillers	Sampled on 7. vi. 23 Total stems	Sampled on 25. vi. 23 Total stems
Supreme	57·0	55·4	56·1	64·2
Victory	59·8	30·7	40·2	59·1
Record	39·3	39·4	39·4	58·7
Leader	63·0	52·1	56·5	61·9
Black Tartarian	53·3	53·3	53·3	64·1
Yielder	60·1	46·3	51·5	64·4
Goldfinder	33·0	41·6	37·2	46·2
Captain	61·2	50·0	56·2	60·3
Sir D. Haig	51·1	54·3	53·0	54·8
Superb	53·3	31·4	43·4	69·9
New Abundance	37·5	50·0	44·0	49·3
Scotch Potato	53·8	49·3	51·3	58·8
Mean percentage	51·9	46·1	48·5	59·6

The mean percentage infestations for main and exposed tillers were 51·9 and 46·1 respectively, the closeness of the values showing that the fly quickly deserted the main stems in favour of the young tillers for oviposition purposes. The increase in the mean infestation of total stems as the result of exposure to infection for 18 days following 7. vi. 23 was only 11·1 per cent.[1] The minimum, mean and maximum records for the hidden tiller infestation were (*a*) June 7, 8·2, 14·9, 21·2 per cent., (*b*) June 25, 6·5, 25·8 and 37·9 per cent. respectively. The range of attack for each size class of stem is shown in Table III, the observations having been made on the later sample.

Table III.

Percentage infestation of stem, sample 25. vi. 23, *Oxford.*

(*a*) Attack including growing point.
(*b*) Attack excluding same.

Length of stem including blades		Minimum	Mean	Maximum
Buds plus hidden tillers		6·5	25·8	37·9
1–6″	(*a*)	58·1	69·9	78·0
	(*b*)	0	1·6	4·7
6–12″	(*a*)	54·7	65·2	80·0
	(*b*)	0	2·3	7·9
12″ and over	(*a*)	11·8	19·9	30·0
	(*b*)	0	2·6	8·8

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[1] A mean increase of 17 per cent. in the total number of exposed stems, available for the fly, was observed by the date of the second sampling.

The attack on exposed stems below 12″ in height, including the longest blade, was three times as intense as that on those above 12″. Of the latter, one-fifth were rendered incapable of producing panicles, while one-quarter of the buds were destroyed, the latter damage being of no economic importance except in so far as it increases the July population of flies.

Comparing the different varieties the minimum, mean and maximum percentages of the stems 12″ and over in length were 10·3, 17·6 and 24·8 and of the buds 16·1, 22·9 and 28·2. The effective attack on the most vigorous stems ranged from 11·8–30·0 per cent. but in relation to the total numbers of stems the range was only 2·0–4·5 per cent.; similarly, in the case of buds, the range of attack was 1·3–9·3 per cent.

It is stated frequently that different varieties of oat have different powers of recovery from infestation, by which is meant presumably that certain varieties yield better than others even though infestation may be apparently evenly distributed. This elusive quality, about which nothing is known, would seem to be dependent (1) on the attack away from the growing point, such stems being still capable of producing panicles, (2) on the early damage to hidden buds and small tillers, on which subsequent tillering depends, and (3) mainly on the capability of the plant to produce effective panicles after the first shoots have been destroyed. The first type of damage has been shown to be negligible (Table III (*b*)), while the second type was variable. Normally the attack on the buds and hidden tillers would have no effect on the yield of the crop, but variation in this type of damage (if early) might be of some importance.

The whole problem of recovery thus remains one of the many problems requiring elucidation.

(*b*) *Grain.*

Shooting of Panicles. The emergence of the panicles from the sheaths was spread over the month of July at Oxford. By July 8 the mean emergence was 8·8 per cent., *Record*, *Black Tartarian*, *Goldfinder* and *Scotch Potato* oats not having started, while *Captain* oats had reached 23·9 per cent. By July 16 the mean emergence was 59 per cent., *Leader* oat having completed emergence while *Sir Douglas Haig* and *Scotch Potato* oats were still over 20 per cent. below the mean. By July 24 *Sir D. Haig* oat was still 15 per cent. below the mean of 75·8 per cent., the remaining varieties closely approximating to the mean.

Although there was great variation in the time of emergence of the panicles, the coefficient of correlation between the percentage emergence by July 16 and the percentage infestation of the grain was only + 0·47 ± 0·15; with the arithmetic mean percentage infestation of stem the coefficient was + 0·28 ± 0·18, showing that the rate of panicle shooting was dependent on varietal differences rather than on intensity of infestation of stem.

The minimum, mean and maximum numbers of seed examined were (*a*) Oxford 560, 665, 721, and (*b*) Harpenden 655, 802 and 935 respectively. The range of variation among the varieties of oat as determined at Oxford is shown in Table IV.

Table IV.

Percentage infestation and "blindness" of grain, Oxford.

	Minimum	Mean	Maximum
Percentage of Bosom Grain	18·5	44·1	50·9
Main Grain, infested	28·3	45·9	55·3
,, "blind"	3·2	7·5	14·3
Bosom Grain, infested	15·1	22·8	30·0
,, "blind"	7·3	13·3	30·7
Total grain, infested + "blind"	31·3	45·6	57·6
"Blind" grain	4·7	9·8	16·6
"Blind" grain, Harpenden	5·2	7·4	8·7

Scotch Potato oat with only 18·5 per cent. of bosom grain was far below the mean. The mean infestation of the main grain was 45·9 per cent. against 22·8 per cent. for the bosom grain, showing that the time factor was of considerable importance here. *Black Tartarian* oat produced the minimum quantity of "blind[1]" main grains (3·7 per cent.), *Abundance* oat producing the maximum quantity (14·3 per cent.). The varieties *Yielder* (30·7 per cent.) and *Captain* (29·3 per cent.) produced "blind" bosom grain in abnormal quantities, *Abundance* being next with 16·4 per cent., and the remainder ranging from 7–11 per cent. only. The mean total production of "blind" grain was considerable, being 9·8 and 7·4 per cent. at Oxford and Harpenden respectively.

In Table I are recorded the arithmetic mean percentage infestations of the grain for the twelve varieties of oats under observation at Oxford and Harpenden, together with the standard errors of the means. It should be noted that, owing to the different methods of sampling, the standard errors of the Oxford means are due to field variations as well as actual errors in sampling, while those of the Harpenden means are due to errors of sampling from a mass of material only.

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[1] As indicated previously, "blindness" in the sense in which the term is used here means that the causal agent was unknown, but was considered not to be the frit fly.

IV. STATISTICAL ANALYSIS OF DATA.

(a) *Coefficients of correlation.*

The mutual relationship between two series of variates is best expressed by the coefficient of correlation (r), which may be determined readily from the formula $r = \frac{\Sigma(d_x d_y)}{n\sigma_x\sigma_y}$, the probable error of r being $\frac{0{\cdot}674(1-r^2)}{\sqrt{n}}$. When r equals or exceeds six times the probable error, correlation between the variates definitely exists and in such cases if r is above 0·5 there is decided correlation. Some of the coefficients of correlation which have been calculated from the experimental data available are recorded in Table V.

Table V.

Coefficients of correlation.

	A. ***Stem.***	
	Arithmetic mean percentage infestation, second sample, Oxford, with	
1.	**Percentage of stunted plants (6″ and less) in samples, Oxford**	**+0·69±0·10**
2.	**Stem infestation, Harpenden ... ...**	**+0·75±0·08**
3.	**Tillering capacity, Oxford ... ...**	**+0·41±0·16**
4.	**Total stems per plant, Oxford ... ...**	**+0·42±0·16**
5.	**12″ stems and over per 100 plants, Oxford ... ...**	**−0·67±0·10**
6.	**6″ ,, ,, ,, ,, ,, ...**	**−0·38±0·17**
7.	**Rate of panicle shooting... ... ...**	**+0·28±0·18**
	B. ***Grain.***	
(*a*)	**Arithmetic mean percentage infestation, Oxford, with**	
1.	**Stem infestation, Oxford ... ...**	**+0·43±0·16**
2.	**"Blind" grain, Oxford ... ...**	**+0·56±0·13**
3.	**Grain infestation, Harpenden ... ...**	**−0·07±0·19**
4.	**Rate of panicle shooting... ... ...**	**+0·47±0·15**
(*b*)	**Arithmetic mean percentage infestation, Harpenden, with**	
1.	**Stem infestation, Harpenden ... ...**	**−0·50±0·14**
2.	**"Blind" grain, Harpenden ... ...**	**+0·11±0·19**
	C.	
1.	**Infested plus "blind" grain, Oxford with Harpenden ...**	**+0·17±0·14**
2.	**Varietal yield values, Oxford with Harpenden,**	
	(*x*) Infested grain only	**+0·67±0·10**
	(*y*) Infested plus "blind" grain	**+0·62±0·12**

There was a decided correlation between infestation and stunting of the plant, but half the stunting was caused by other factors, *i.e.* our knowledge of the percentage infestation only gives as much information about the percentage of plants stunted as all other independent factors put together. Tillering capacity was not definitely correlated with infestation. The percentage increase between June 7 and June 25 in the number of exposed tillers ranged from 6–124 with a mean of 44, but this additional tillering was not correlated either with main stem infestation ($r = -0{\cdot}006 \pm 0{\cdot}21$) or with exposed stem infestation definitely

$$(r = +0{\cdot}44 \pm 0{\cdot}17),$$

as determined on June 7. On the other hand, it is a matter of observation that a plant losing the main stem early shows advanced tillering in many cases. The sap supply to the damaged stem must be reduced considerably, although not entirely inhibited, the greater part of the material being available elsewhere. The production of stems 12″ and over in height showed a decided negative correlation with infestation, as was to be expected. Correlation between the stem infestations at Oxford and Harpenden was very high ($+0{\cdot}75 \pm 0{\cdot}08$), showing that variation in locality alone should not markedly affect the relative variations in stem infestation.

The position with regard to the grain was very different. The grain and stem infestations in the one locality showed no certain correlation, while the grain infestations at Oxford and Harpenden showed no correlation whatever ($-0{\cdot}068 \pm 0{\cdot}19$). The independence of the intensities of infestation of the stem and grain indicates that the grain infestation is very dependent on a time factor. As the production of "blind" grain was not correlated with infestation, it would appear that very few errors from this source were included with the estimates of frit infestation.

(*b*) *Varietal differences in intensity of infestation.*

Certain differences in intensity of infestation between the varieties of oats used during this investigation have been recorded for both stem and grain. The value of records of this type is greatly increased if they are subjected to statistical analysis (1) to determine whether any two values are significantly different or whether the observed differences may be ascribed to fluctuations of sampling, and (2) to combine the evidence derived from independent experiments. A short description of the formulae utilised may be useful to those unacquainted with statistical methods as they are generally applicable and are valuable aids in deducing conservative conclusions.

To determine whether two values x and y of arithmetic mean percentage infestation in the same series are significantly different (*i.e.* whether the observed difference in infestation is independent of fluctuations of sampling) it is necessary to divide the observed difference (D) between the arithmetic mean percentage infestations by the standard error of the difference ϵ_{xy} obtained from the formula $\epsilon_{xy}^2 = \frac{\sigma_x^2}{n_x} + \frac{\sigma_y^2}{n_y}$, where σ^2 equals $\frac{\Sigma d^2}{n-1}$. If $\frac{D}{\epsilon_{xy}}$ equals or exceeds 2·57, then the chances are 100 to 1 or more that the observed difference is significant and not due to fluctuations of sampling.

The values obtained independently at Oxford and Harpenden have been combined[1] by means of the corresponding standard errors from the formula

$$X = \frac{\frac{D_{\mathrm{O}}}{\epsilon_{\mathrm{O}}^2} + \frac{D_{\mathrm{H}}}{\epsilon_{\mathrm{H}}^2}}{\frac{1}{\epsilon_{\mathrm{O}}^2} + \frac{1}{\epsilon_{\mathrm{H}}^2}},$$

where X is the mean precentage difference in infestation between two varieties weighted according to the independent observations, D the

Table VI.

Stem analysis.

O = Oxford H = Harpenden

Variety		Difference between arithmetic mean percentage infestations (D)	Standard error of $D = \epsilon_{xy}$	$\frac{D}{\epsilon_{xy}}$	Weighted mean difference in percentage infestation (X)	Standard error (E) of X	$\frac{X}{E}$
Supreme	O	18·3	4·7	3·88	17·2	3·9	4·36
	H	14·6	7·2	2·02			
Superb	O	22·2	6·8	3·28	17·2	5·0	3·46
	H	11·6	7·2	1·60			
Yielder	O	19·1	4·9	3·87	16·4	4·1	3·99
	H	10·1	7·5	1·36			
Black Tartarian	O	19·7	5·5	3·58	15·1	4·4	3·46
	H	7·1	7·2	1·00			
Captain	O	14·2	5·3	2·67	12·9	4·3	3·01
	H	9·5	7·2	1·31			
Victory	O	11·9	4·2	2·83	10·5	3·6	2·90
	H	6·9	7·1	0·97			
Record	O	12·1	7·8	1·56	13·4	5·2	2·56
	H	14·5	7·1	2·04			
Leader	O	11·6	6·1	1·90	11·1	4·6	2·41
	H	10·4	7·1	1·48			
Scotch Potato	O	9·9	6·4	1·55	8·0	4·9	1·63
	H	5·4	7·6	0·71			
Sir D. Haig	O	8·3	7·0	1·18	7·8	5·3	1·44
	H	7·2	8·3	0·87			
New Abundance	O	2·2	7·1	0·31	1·9	5·3	0·36
	H	1·5	7·9	0·19			
Goldfinder	Variety with the minimum arithmetic mean percentage infestation, with which the remainder have been compared.						

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[1] We are indebted to Mr Fisher for this method of calculating the weighted mean.

Table VII.

Grain analysis.

O = Oxford H = Harpenden

Variety		Difference between arithmetic mean percentage infestations (D)	Standard error of $D = \epsilon_{xy}$	$\frac{D}{\epsilon_{xy}}$	Weighted mean difference in percentage infestation (X)	Standard error (E) of X	$\frac{X}{E}$
New Abundance	O	8·2	3·5	2·32	14·3	1·75	8·2
	H	15·5	2·0	7·92			
Sir D. Haig	O	5·5	3·6	1·53	8·3	1·54	5·4
	H	9·0	1·7	5·30			
Black Tartarian	O	8·4	2·9	2·86	7·5	1·22	6·3
	H	7·3	1·3	5·40			
Yielder	O	15·4	4·0	3·81	5·4	1·90	2·8
	H	2·6	2·1	1·21			
Victory	O	6·9	3·0	2·33	4·6	1·19	3·9
	H	3·6	1·2	2·98			
Leader	O	11·1	3·5	3·18	4·6	1·71	2·7
	H	2·5	2·0	1·28			
Supreme	O	15·7	3·7	4·20	4·0	1·23	3·3
	H	2·6	1·3	2·00			
Goldfinder	O	10·0	2·8	3·68	3·6	1·17	3·0
	H	2·2	1·3	1·71			
Captain	O	11·4	4·7	2·44	2·7	1·19	2·3
	H	2·1	1·2	1·71			
Superb	O	11·3	2·9	3·85	2·4	1·29	1·9
	H	0·3	1·4	0·21			
Record	O	8·0	3·6	2·21	1·3	1·17	1·1
	H	0·5	1·2	0·40			
Potato	Variety with the minimum arithmetic mean percentage infestation, with which the remainder have been compared.						

observed difference between the arithmetic mean percentage infestations, and ϵ the standard error, denoted above as ϵ_{xy}. The standard error (E) of X is $\frac{1}{\sqrt{1/\epsilon_O^2 + 1/\epsilon_H^2}}$. The value of the weighted mean difference in mean percentage infestation between two varieties may be tested by the relation of X to E as indicated above.

The results of such analyses for both stem and grain infestations are shown in Tables VI and VII and the importance of this method of examining the results of observation is exemplified by the two cases of grain infestation, *Leader* and *Captain*, at Oxford. In addition two independent values, each of doubtful significance alone, may reinforce one another, giving a significant weighted mean value, *e.g.* Table VI, *Record*.

The coefficients of variability indicate that the variation between parallel samples was distinctly lower in general at Harpenden than at Oxford, but *Goldfinder* at Harpenden was exceptional in this respect, thereby increasing the Harpenden standard errors and somewhat reducing the weight of the Harpenden data in the weighted means for the stem infestation.

V. INTERPRETATION OF DATA.

Taking *Supreme* oats as an example to explain the meaning of the values recorded in Tables VI and VII, the weighted mean difference in percentage infestation of stem from the value observed for *Goldfinder* oats was 17·2 ± 3·9. The figure 17·2 is the *most probable value* of the difference and is assumed to be the true value under the varied conditions of both series of experiments. Both + and – deviations are equally likely to occur in other series of experiments, but less and less frequently as they increase in magnitude, while the chances are about 330 to 1 that most of the deviations will lie within the limits of the mean ± three times the standard error.

Stem. Supreme, Superb, Yielder, Black Tartarian, Captain and *Victory* oats, in this order, suffered from 17·2–10·5 per cent. greater stem infestation than *Scotch Potato, Sir Douglas Haig, New Abundance* and *Goldfinder* oats. The latter form a group in which the differences were not significant under the conditions of these experiments. *Record* oat should probably be included with the first series, while the position of *Leader* oat is doubtful[1].

With regard to the pressed samples of the several varieties, a careful examination has up to the present failed to show any differences in growth, tillering, etc. which can be correlated with the observed differences in extent of infestation, even in the cases of the extremes. At the same time, this line of investigation has not been carried as far as is desirable and as additional material accumulates, the above observation may have to be modified.

Grain. With regard to the grain infestation the group suffering least infestation included *Captain, Superb, Record* and *Scotch Potato* oats. *Victory, Leader, Supreme* and *Goldfinder* oats suffered less than 5 per cent. heavier infestation; *Sir Douglas Haig, Black Tartarian* and *Yielder* oats less than 10 per cent. but more than 5 per cent. heavier infestation; while

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[1] Mr Fisher has calculated the weighted average stem infestations, comparing the varieties with the mean infestation of 62·45 per cent., with the result that *Supreme* (68·62 per cent.) and *Superb* (68·17 per cent.) are again shown to be significantly highly infested and *New Abundance* (54·41 per cent.) and *Goldfinder* (52·65 per cent.) significantly low.

New Abundance was decidedly the heaviest sufferer, showing an infestation 14·3 per cent. above that of *Scotch Potato.*

The range of the differences was of the same order in both stem and grain infestations.

Comparative Yield Values. Yield of straw is dependent on (1) the percentage of uninfested stems, and (2) the tillering capacity of the plants, particularly when subjected to infestation. No observations have been made in relation to this subject.

Yield of grain is dependent on (1) the percentage of clean stems, (2) the percentage of clean seeds borne by the clean stems, (3) the extent of material damage to the seed, which will probably be a more or less constant factor for all varieties, and (4) the average size and weight of the clean seed of the variety. Therefore by taking both stem and grain infestations into consideration values representing percentage yield of unattacked grain may be calculated and compared. These values are shown in Table VIII, column A being based on the percentage of grain free from frit infestation and column B on the percentage of grain free from both frit infestation and blindness. Thus assuming the maximum yield to have been 100 seeds, Table VIII shows the relative number of clean seeds produced by each variety under the conditions of these experiments. The Oxford and Harpenden values thus obtained have a coefficient of correlation of + 0·67 ± 0·10.

Table VIII.

Comparative Varietal Yield Values.

A. Based on percentage of frit-free grain.

B. Based on percentage of unattacked developed grain*.

Variety	Oxford		Harpenden	
	A	B	A	B
Goldfinder	34·2 ± 2·5	29·6	36·7 ± 6·3	33·0
New Abundance	33·6 ± 4·2	25·7	29·8 ± 3·4	26·4
Scotch Potato	32·3 ± 4·0	30·2	33·0 ± 3·5	30·2
Sir D. Haig	30·8 ± 4·3	27·4	28·1 ± 4·2	25·3
Victory	27·8 ± 1·6	24·4	30·3 ± 2·2	27·9
Record	27·4 ± 4·6	23·7	25·0 ± 2·3	23·2
Leader	26·3 ± 3·2	22·7	27·8 ± 2·2	26·3
Captain	24·5 ± 2·9	18·1	28·7 ± 2·6	25·8
Black Tartarian	22·2 ± 2·7	20·4	28·0 ± 2·3	26·6
Supreme	20·5 ± 2·0	17·6	24·3 ± 2·5	22·1
Yielder	20·2 ± 2·3	14·8	28·1 ± 3·1	25·4
Superb	19·6 ± 3·6	17·0	27·6 ± 2·6	25·3

* The standard deviations of the mean values recorded in columns B have not been calculated, but will be of the same order as those of the respective A columns.

Tested by the relation of the percentage difference (D) to the standard deviation (ϵ_{xy}), none of the Harpenden percentage differences are significant among themselves, owing to the relatively great variability of the stem infestation in *Goldfinder* oat. Approximations to the standard deviations of the yield values have been calculated from the formula

$$\sigma^2_{\text{Stem} \times \text{Grain}} = M_S^2 \sigma_G^2 + M_G^2 \sigma_S^2,$$

and weighted mean yield values then obtained by the method previously indicated, these values being recorded in Table IX.

Table IX.

Analysis of yield of frit-free grain.

Variety	Weighted mean difference in percentage yields	$\frac{X}{E}$
Goldfinder	Variety with maximum yield value, with which the remainder have been compared	
Scotch Potato	− 2·4±3·9	0·62
New Abundance	− 2·5±4·1	0·62
Sir Douglas Haig	− 4·9±4·1	1·19
Victory	− 6·4±2·7	2·40
Leader	− 8·0±3·4	2·35
Record	− 8·7±4·1	2·13
Captain	− 9·3±3·3	2·80
Black Tartarian	−11·4±3·2	3·55
Yielder	−13·1±3·0	4·32
Superb	−13·1±3·6	3·59
Supreme	−13·4±3·2	3·55

The varieties *Goldfinder*, *Scotch Potato*, *New Abundance*, *Sir Douglas Haig*, *Victory*, *Leader* and *Record* form a group in which the observed differences in percentage yield of frit-free grain may be ascribed to fluctuations of sampling. *Captain*, *Black Tartarian*, *Yielder*, *Superb* and *Supreme* oats showed significant negative differences, varying from 9·3–13·4 per cent. respectively, when compared with *Goldfinder* oat. The latter variety had a percentage yield value of unattacked developed grain equal to 35 per cent. only of the total yield possible under the conditions of the experiments, the observed differences between the two groups therefore being of importance.

VI. SUMMARY.

Twelve varieties of oat (*Supreme*, *Victory*, *Record*, *Leader*, *Black Tartarian*, *Yielder*, *Goldfinder*, *Captain*, *Sir Douglas Haig*, *Superb*, *New Abundance* and *Scotch Potato*) have been grown in widely separated localities, namely, Sandford, Oxon., and Harpenden, Herts., where they were subject to the attack of *Oscinella frit*, L. The infestations of stem and grain were observed in detail and the values obtained subjected to statistical analysis.

1. The stem infestation showed an increase of 11 per cent. only, 18 days after June 7.

2. The attack on stems 12″ in length (including blades) and over was only one-third as intense as that on the remaining stems; 20 per cent. of the former were rendered incapable of producing panicles; 25 per cent. of the hidden buds plus small tillers were destroyed. Infestation away from the growing point averaged 2 per cent.

3. The infestation of the main grain reached 45·9 per cent. against 22·8 per cent. for the bosom grain, at Oxford, indicating the importance of the time factor.

4. The production of "blind" glumes (*i.e.* those containing undeveloped seed, causal agent unknown) was considerable (7–9 per cent.), but independent of frit infestation of grain.

5. The correlation between stem infestations at Oxford and Harpenden was high, but the grain infestations showed no correlation whatever; neither was there any definite evidence of correlation between stem and grain infestations in either locality. No correlation between infestation and tillering capacity was discovered, but the production of vigorous stems was decidedly correlated with the absence of infestation.

6. Weighted mean differences in mean percentage infestations both for stem and grain are recorded for each variety, comparisons being made with the *Goldfinder* oat in the former case and the *Scotch Potato* oat in the latter case.

7. With regard to the stem infestation, *Scotch Potato, Sir Douglas Haig, New Abundance* and *Goldfinder* oats were proved, under the conditions of these experiments, to be least liable to infestation, but it was not possible to differentiate between them. The remaining varieties suffered from 10–17 per cent. heavier stem infestation, the differences observed being significant.

8. With regard to the grain infestation, *Captain, Superb, Record* and *Scotch Potato* oats suffered the least infestation, *New Abundance* oat being most heavily infested.

9. Considering the percentage yields of unattacked developed grain, *Captain, Black Tartarian, Yielder, Superb* and *Supreme* oats, when compared with *Goldfinder* oat, were found to yield less by 9·3–13·4 per cent., the yield of *Goldfinder* oat being equal to only 35 per cent. of the total yield possible under the conditions of this experiment.

REFERENCES.

(1) FRYER, J. C. F. and COLLIN, E. (1924). Certain Aspects of the Damage to Oats by the Frit Fly. *Ann. App. Biol.* Vol. XI, Nos. 3 and 4.

(2) HARPER GRAY, R. A. (1923). Frit fly on oats in the four northern counties. *Journ. Min. Agric.* March, 1109–14.

(3) KLEINE, R. (1923). Die Anfälligkeit bzw. Widerstandsfähigkeit einzelner Hafersorten gegen den Befall durch *O. frit*, Linn. *Zeitschr. Schädlingsbekämpfung*, I, No. 1, 2–12.

(4) PETHERBRIDGE, F. R. (1924). The common cause of failure of spring oats—frit fly. *Jour. Min. Agric.* XXX. 925–928.

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THE APPLE BUD WEEVIL, *ANTHONOMUS CINCTUS*, KOLL.

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(With Plate XVI and 10 Text-figures.)

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1. Introduction.

In the spring of 1921 complaints were received from Mr W. G. Kent, the Horticultural Superintendent of the Isle of Ely County Council, that apple fruit buds were being killed by a weevil very similar to the Apple Blossom weevil (*A. pomorum*). This damage was first noticed by Mr H. J. Gardner of Wisbech whilst pruning cordons in March. On April 8th we visited Messrs Gardner Bros. and brought back a few of the weevil larvae in the buds they had hollowed out.

From the resemblance of these to the larvae of *A. pomorum* we formed the opinion that the abnormally warm conditions prevailing in this district in the early part of 1921 had caused *A. pomorum* to lay their eggs very early; and, as no blossoms were available, we presumed they had laid them on the blossom buds. At the beginning of May the larvae pupated and early in June weevils hatched out. These were identified by Messrs Champion and Harwood (P.) as *Anthonomus cinctus*, Koll. On August 14th, 1921, a single specimen of this weevil was found, in company with *A. pomorum*, under apple bark in an orchard adjoining Darenth Wood (in Kent) by Mr P. Harwood (1).

In the spring of 1922 injured fruit buds containing weevil larvae were found in two other portions of Messrs Gardners' orchards, situated more than 100 yards from the cordons where the weevils were found in 1921.

A search was made in the neighbourhood of Cambridge for buds containing this weevil and, on May 14th, 1922, we found three apple buds hollowed out, each containing a bud weevil larva, in an orchard near Histon. Young pear trees situated about 200 yards from the infected apples showed no sign of attack, although on the Continent this insect is regarded as a pear pest.

Later in the season, further evidence of this pest in another orchard (nearly two miles from the one previously mentioned) was afforded by the presence of dead hollowed-out buds with the characteristic exit hole of the weevil (see Plate XVI, fig. 3). On November 8th bud weevils were found on the "tanglefoot" bands placed round the trunks of these trees.

2. SYNONYMS.

Anthonomus cinctus Kollar, Redtb. Fauna 2. 309.
Anthonomus pyri Boh. Schonherr Gen. and Spec. Curculionidum 7. 2. 215.
Anthonomus bituberculatus Thoms. Skandinaviens Coleoptera, 1868.

This species should not be confused with *A. cinctus* Thoms. = *A. inversus* Bedel = *A. ulmi* Debr. (non Deg.) and British Catalogue. Von Frauenfeld, *Verh. z. b. Ges. Wien*, 1872, p. 393, describes under the name of *A. cinctus* Sch. a species which lives on *Pyrus salicifolius*. The identity of this insect has not been determined (Bedel) (2).

3. DESCRIPTION.

Adult (see Fig. 1).

Length (including rostrum) 4·5–5·0 mm.

Brownish-red or purplish-red, clothed with white, golden and black pubescence.

Head with two tufts of white pubescence between the large prominent eyes.

Rostrum long, slightly curved, rugose and ridged, apex and sides black.

Thorax with numerous coarse punctures, clothed with golden and black pubescence, with a line of coarse white pubescence running down the centre.

Scutellum small, with dense coarse white pubescence.

Elytra covered with white, golden and black pubescence and having a marked transverse fascia of white pubescence behind the middle, broader at the margins, bordered in front and behind by black hairs; *bearing a tubercle near the base of the third interstice densely covered with black hairs* (in addition to the shoulder characteristic of Anthonomina). Interstices wide and rough. Striae coarsely punctured.

Legs reddish-brown with black patches in places but very variable. Anterior femora with a very strong tooth. Basal half of anterior tibiae strongly sinuate on inner margin, other tibiae almost straight, but bent at base.

External Sexual Differences. The sexes can be distinguished by examination of the ventral surfaces, without injury to the specimens. In the *male* the posterior margin of the pygidium is folded into a definite ridge forming an angle of about 90° at the apex, the angle increasing towards the base. The ventral side of the ridge is smooth and shining: the dorsal surface of the ridge is covered with white hairs. (Apart from the ridge the pygidium is markedly convex.) In the female the posterior margin of the pygidium is not folded, but is markedly convex.

There are also slight differences in the rostra. In the *male* the rostrum is shorter, narrower at the base, and gradually enlarging to the apex. In the *female* the rostrum is longer, thicker at the base and enlarging from the point of insertion of the antennae to the apex.

Plate XVI, fig. 2 shows the male genitalia, and may prove useful in distinguishing it from allied species.

Distinction from Allied Species.

A. cinctus resembles fairly closely some of the other species of *Anthonomus*. It most closely resembles *A. ulmi* and may be present in some collections under this name, as it runs down to this species in Fowler's key better than *A. ulmi* itself.

A. cinctus (Koll.) Larger	*A. ulmi* (De G.) Smaller
Reddish-brown with a purplish tint, darker than *A. ulmi*.	Brownish-red (very variable).
Tubercle near the base of the 3rd interstice of each elytron densely covered with black hair.	Small tubercles *sometimes* present near base of 3rd interstice of each elytron; fewer black hairs.
Rostrum longer, 1·4–1·6 mm.	Rostrum shorter, 1·0–1·35 mm.
Antennae longer, 1·95–2·05 mm.	Antennae shorter, 1·3–1·4 mm.
Thorax relatively wider, about 1·33–1·4 times the length.	Thorax relatively narrower, from about 1·2–1·3 times the length.

Fowler says of *A. ulmi*, De G. "Elytra with a little oblong tubercle or small prominence at the base of the third interstice," but he adds later, "the points of difference which have usually been given as separating it from the following (species) are sometimes hard to make out satisfactorily."

Reitter (3) says of *A. inversus*, Bedel (= *A. ulmi*, De G.), third interstice not broadened, rarely raised and with no black hairy protuberance.

A. pedicularius, L. can be distinguished from *A. cinctus* by the following characters. Absence of tubercle on 3rd interstice, a much shorter rostrum, much straighter and scarcely sinuate anterior tibiae, smaller tooth on anterior femora and in fresh specimens by the presence of two white fasciae on the elytra, the anterior of which is not so marked.

A. chevrolati, Desb. can be distinguished from *A. cinctus* by the absence of a tubercle on the third interstice, but right at the basal margin the extreme base of the third interstice is raised.

A. cinctus is easily distinguished from *A. pomorum* which is smaller, darker in colour, and has a strongly oblique white fascia on each elytron forming a V-shaped marking.

The Egg.

Time of Egg-laying. Most of the continental observers agree that eggs are laid in pear buds in the autumn. Boisduval (4) and Taschenberg (5) state that eggs are laid in the spring.

In 1922 none of our "sleeved" weevils laid eggs, but on February 3rd, 1923 we found eggs in apple buds near Histon, and were unable to find adults on or after this date. In 1923 all our "sleeved" weevils except two died without laying eggs. These two, one female and one male, were "re-sleeved" on October 24th, and on November 5th one egg was found. Another egg was laid between January 3rd and January 23rd, 1924. The male was dead on February 28th, and the female on March 6th.

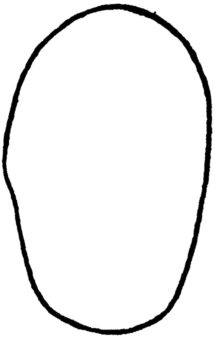


Fig. 1. Egg of *A. cinctus*.

Position. The eggs are laid in the bud of the apple; and, in a very large percentage of cases, in the fruit buds. A hole is made in the bud by the female (see Plate XVI, fig. 3) usually just below or near the equator, and the egg is placed at the bottom of the burrow. The egg usually has two bud-scales immediately outside it, but it may be deeper, or underneath the outer bud-scale only. We never found more than one egg in a bud.

Structure. The egg measures 0·7–0·8 mm. in length, and 0·5–0·59 mm. in width. It is of a creamy-white colour, and smooth, without any distinctive markings. It is bean-shaped, being broader at the head end than at the distal end. Its shape is shown in Fig. 1.

Just before hatching the head darkens and can be seen through the transparent chitin; the proximal end of the egg for about two-thirds of its length is more opaque than the rest, and white longitudinal marks (apparently the fat bodies) can be seen.

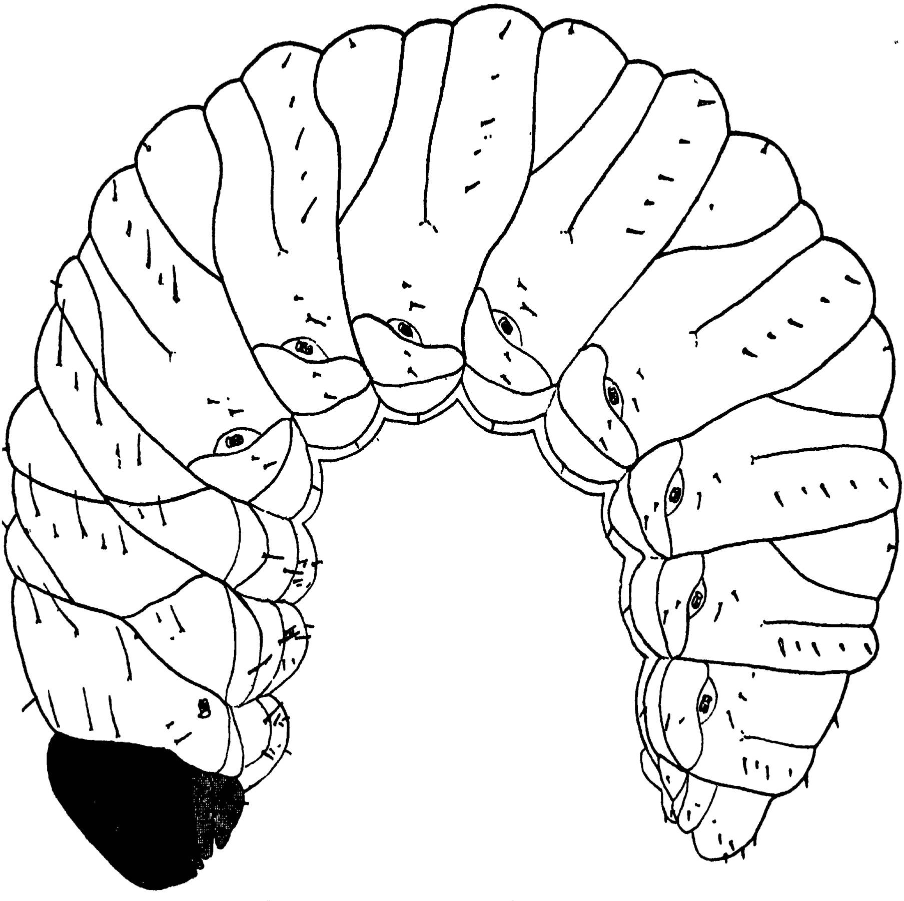


Fig. 2. *Anthonomus cinctus.* Third stage larva.

The distal end is somewhat transparent and dusky.

1st Stage Larva. Length in normal curled condition 0·9–1·0 mm.
Width of head 0·35 mm.
Spiracle. Length about 25μ, width 15μ.

This larva differs from the 3rd stage larva described below in the comparatively large size of the head, which is also lighter in colour. The mandibles have a distinct file-like serration on the lower edges of their two teeth, and the segments are very much more difficult to differentiate.

2nd Stage Larva. Length in normal curled condition about 1·8 mm.
Width of head 0·46 mm.
Spiracle. Length about 32 μ, width 22 μ.

This larva is intermediate in characteristics between the 1st and 3rd stages.

3rd Stage Larva. (See Fig. 2.) Length in normal curled position about 3·5 mm. (True length about 5·5 mm.)
Width of head ·55–·6 mm.

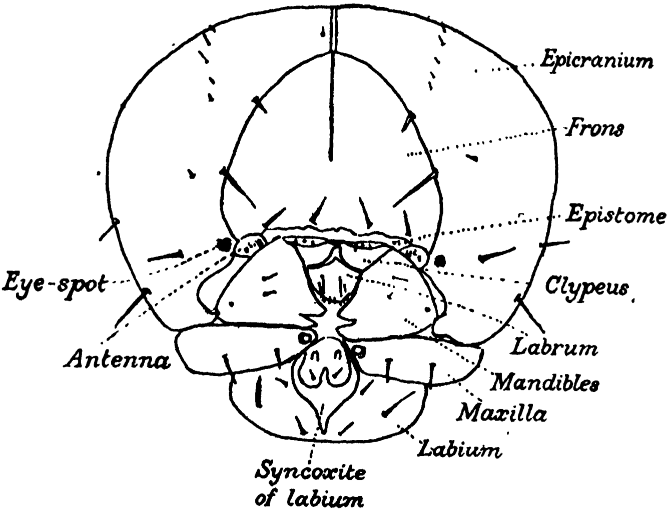


Fig. 3. Head of third stage larva of *A. cinctus*.

It resembles the larva of *A. pomorum* but is broader and more curled. The head is dark brown and fairly large. The body is creamy-white, soft and fleshy and with numerous transverse wrinkles. The skin is transparent and the outlines of the cream-coloured lobules of the fat bodies alternating with the darker colour of the body contents give the larva a characteristic appearance. The dorsal vessel is plainly visible as a brownish line down the centre of the dorsal surface and its pulsations can be seen under the lens.

The head (Fig. 3). The epicranium is divided down the middle by a suture and bears several longish setae, two pairs along the arms of the epicranial suture and three pairs a little behind the anterior angles of the head. There are also five minute setae on either side of the vertex.

The sclerite formed of the fused frons and epistome is subcordate, with two setae on each side, one near the base of each antenna, and one on either side of the median line, together forming a transverse row at the anterior margin of the epistome. A marked sagittal furrow extends half-way from the vertex towards the anterior margin.

The anterior margin is thickened, and somewhat waved, and bears two pairs of small setae and two pits.

The clypeus and labrum together form a sub-triangular plate above the mandibles. The anterior border of the clypeus is emarginate. The labrum bears 16 setae pointing anteriorly and 2 pits. A conical projection from the labrum extends beneath the clypeus (see Fig. 4).

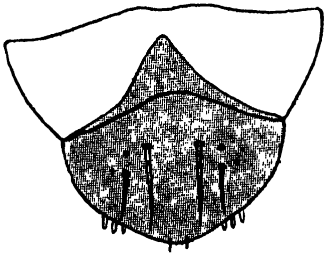


Fig. 4. Labium and clypeus of larva of *A. cinctus.*

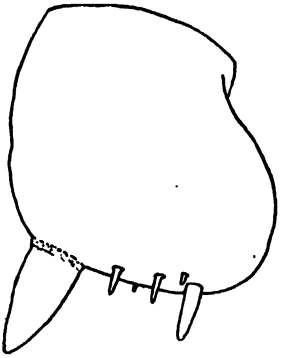


Fig. 5. Left antenna of third stage larva of *A. cinctus.*

The antennae (see Fig. 5) are pale-coloured and situated at the basal angle of the frons. Each is sub-globose, with a bullet-shaped process on the inner side and five smaller processes of similar shape on the outer side.

The *eyes* are represented on the inner margin of the epicranium by a dark pigmented spot just behind the base of the mandibles.

The *mandibles* (see Fig. 6) are stout with two bluntly-pointed teeth of equal size, dark ochreous-brown with the file-like serration on the inner edges of the teeth present in the other two stages, indistinct. Each bears two setae near the centre and a pit near the mandibular condyle.

The maxillae and labium (see Figs. 7 and 8) are prominent on the ventral surface. The maxillary cardo is small and has a bluntly-pointed process on the outer side of the base. The stipes is long, the surface of the

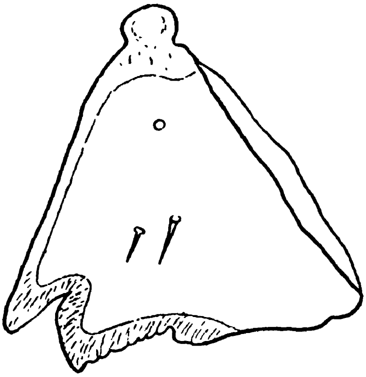


Fig. 6. Mandible of larva of *A. cinctus.*

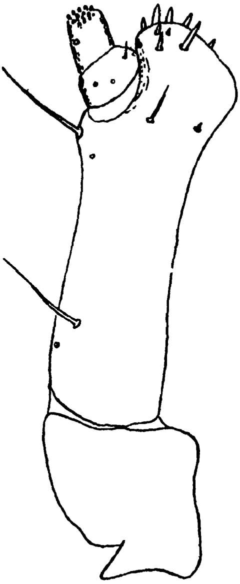


Fig. 7. Maxilla of larva of *A. cinctus.*

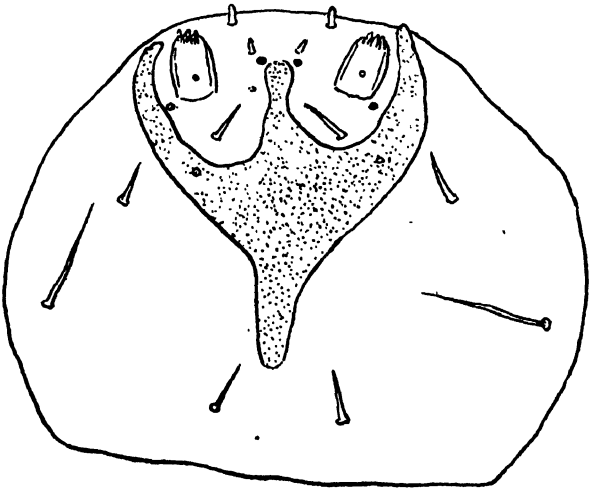


Fig. 8. Labium of larva of *A. cinctus.*

N.B. All these figures are drawn from a third stage larva.

mala bears a small sensory pit on its outer margin and nine hair-like papillae apically. The two-jointed palp has fifteen conical papillae on its disc-like apex and a small seta on its basal segment.

In addition there are on the stipes four setae, one external, close to the cardo, and two near the base of the palp, and one small one almost in line on the inner margin.

The base of the labium is composed of a fused membranous submentomentum which bears three pairs of setae. The terminal lobe (the syncoxite of Verhoeff (6)) is rounded and has a brown chitinous trident-shaped support (see Fig. 8). There are several pairs of setae and sensory tubercles between its arms. The single jointed palps are situated near the anterior border and each bears eight or nine small conical pegs at its apex.

Thorax and Abdomen. The lateral longitudinal folds are clearly seen dividing the body into epipleural, hypopleural and ventral regions.

The folds bear brownish setae of two sizes, arising from small tubercles, and the longer and shorter ones alternate. The body is clothed with very small evenly-spaced papillae.

The Thorax. On each side of the prothorax are four setae near the anterior, three near the posterior margin, two near the spiracle and two on the hypopleural lobe. On the meso- and meta-thorax there is one seta on the praescutum and five on the scutellum on each side. Laterally each epipleural and hypopleural lobe has one seta and each scutum has two lateral setae. The crural pads of the three thoracic segments each bear six small setae.

The Abdomen consists of ten segments but the tenth is very small and often withdrawn. The first eight abdominal tergites bear setae similar to those on the meso- and meta-thoracic tergites and each scutum has two lateral setae. On the epipleural lobes two setae are present just below the spiracle, and one on each hypopleural lobe.

The ninth abdominal segment bears a few small setae.

Spiracles (see Plate XVI, fig. 4). There are nine pairs of spiracles, one on the posterior edge of the prothoracic tergite and one on each of the first eight abdominal segments. These are situated in depressions just above the epipleural lobes and are hyaline with a slight yellowish tint. They are all nearly equal in size. Length about 58 μ, width 35 μ. They communicate with a two-lobed sac which bears characteristic internal ridges of chitin. The tracheal orifice is circular.

The abdominal spiracles lie parallel to the pleural folds with the tracheal openings in front; while the thoracic ones are at an angle of about 30° so that the tracheal openings are ventral to the sacs.

Pupation.

Just before pupation the larva straightens itself. The dorsal vessel becomes invisible. The ventral surface becomes flattened and the segments and wrinkles indistinct.

The Pupa (Fig. 9) is soft, pale cream in colour. The head is bent beneath the prothorax and carries no bristles. The thorax is the largest segment and its notum carries a number of short bristles each on a conical tubercle. The mesonotum has on either side of the centre a protuberance carrying a group of four bristles. In the middle line there is a round swelling near the base of the segment.

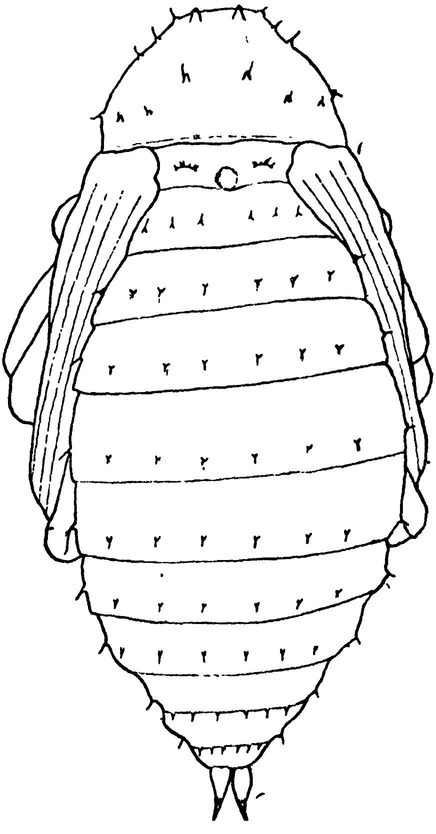


Fig. 9. Pupa of *A. cinctus*.

The metanotum carries three pairs of bristles each on a conical tubercle; and, about the centre, has a triangular depression wider in front.

The ninth abdominal segment bears a pair of stout caudal spines, much swollen at their bases. A small tenth abdominal segment is visible when viewed from the ventral side. On each of the first nine abdominal segments, four pairs of short bristles arising from small conical tubercles are visible from above. Length about 5 mm.

4. DISTRIBUTION AND HOSTS.

This pest is widely distributed on the Continent, having been recorded from Holland, France, Germany, Sweden, Italy, Austria, Hungary and Russia. It is not, however, a very serious pest except occasionally in certain districts.

Dr L. Reh (Hamburg) (*in litt.*, 1924) says, "*Anthonomus cinctus* is almost unknown in Germany."

Dr P. Marchal (Paris) (*in litt.*, 1924) says, "*Anthonomus pyri*, Boh. is often found in France and some seasons does considerable damage in various fruit districts of this country. In general, however, its damage is not widespread. It is particularly troublesome in the valley of the Loire."

In France it is called "The winter worm" and also "The bud worm." In Germany it is called "The pear bud killer" or "The pear bud stinger." There its chief host appears to be the pear, as some observers do not mention it as an apple pest. According to Bedel (2) it is found in orchards and nurseries, and the larva lives in the buds of pear trees. Reitter (3) says, "In blossoming apples and pears." Decaux (7) says, "On pears mostly but found on apples in Picardy and near Paris." Ritzema Bos (8) obtained it from apple buds (in Holland) in 1915 and regards this as its first record from that source.

There is no record of this pest in this country up to 1921 when larvae were found in March damaging the buds of apples at Messrs Gardners, Wisbech, and from which adults were reared in June. Since then P. Harwood (1) records a single adult from Darenth Wood, Kent. Damage and specimens similar to the above were found at Messrs Gardners, Wisbech in 1922 and 1923.

In May, 1922 and again in 1923 we found larvae attacking the buds of apples in an orchard near Histon, Cambs. In 1922 Mr H. Goude (Horticultural Instructor for Norfolk) found specimens causing damage at Tilney in Norfolk.

On November 8th, 1922 we found several adults on "tanglefoot" bands on apple trees in an orchard near Cambridge, and on the same trees we found eggs at the beginning of the following February and later larvae.

In December, 1922, Duffield records two specimens as being found in company with *A. pomorum* by Miss Worsley at East Malling, Kent.

Although this pest is found mainly on pear trees on the Continent, there is no record of it causing damage to, or being found on them in this country.

That this species has been found near Cambridge and Wisbech, in Norfolk, and at two places in Kent, points to the probability of its existence in this country for a number of years rather than to its recent introduction.

In spite of the fact that no collector has recorded its presence until recently (it is possible that it may be present in some collections as *A. ulmi*) it may have been present in small numbers and escaped observation. In support of this, we have been unable, even after careful search, to find this species during the winter 1923–4 on the trees near Cambridge from which we previously collected nearly fifty specimens.

This species is usually present together with *A. pomorum* and a few specimens might easily be overlooked amongst a large number of the latter.

5. NOTES ON LIFE-HISTORY.

In 1921 3rd stage larvae were found in hollowed-out buds on apple cordons at Wisbech. These buds were very noticeable as they had not increased in size, as had the normal buds. Several larvae were brought back to Cambridge and kept in the laboratory where they pupated before May 2nd. At the beginning of June they had all reached the adult stage.

In 1922 further damage by this weevil was found at Wisbech and in an orchard near Cambridge. Larvae were found as late as May 14th. On June 12th eleven beetles sent from Wisbech were "sleeved" on young apple trees and periodically examined in order to try and find out when egg-laying takes place. The "sleeves" were moved at intervals and the buds on the shoots where the weevils had been sleeved were carefully dissected. Several of the weevils died during the summer, but two of them lived until the end of October. No eggs were found.

The following February we began to search for the eggs on those trees where the pest had previously been found. On February 3rd, 1923 we found eggs in the buds of apple trees near those on which we had previously found adults. The presence of last year's hollowed-out buds is useful in indicating which trees are likely to yield results in the search for eggs.

The buds which contain eggs are punctured by a small hole usually near the equator, but this is not always easy to see except by careful examination, owing to the dark colour of the outer bud-scales (see Plate XVI, fig. 3). Some buds were pierced in this way but contained no eggs.

The buds attacked are nearly always fruit buds, but we found two shoot buds also attacked.

Shoots with buds containing eggs were obtained from trees near Cambridge and kept under observation in the laboratory in order to determine the time spent in the various stages and also in rearing any parasites which might be present.

In the laboratory the first larva hatched on February 9th, *i.e.* six days after being brought in. On the trees the first larva was found on February 26th, and the last egg on March 16th.

The following table gives a summary of the life-history during 1923 as found in the orchard.

	When first found	When last found	Time taken in each stage
Egg	Feb. 3rd, but probably was laid much earlier	March 16th	?
1st stage	Feb. 26th	April 7th	3–3½ weeks
2nd stage	March 23rd	April 30th	2–3 weeks
3rd stage	April 6th	May 21st	3–4 weeks
Pupa	May 4th	June 18th	About 4 weeks

As soon as the larva hatches it begins to feed on the folded leaves of the bud. It lives through the larval stages in one bud, eating all the succulent portion and occasionally the softer portion of the stem below the bud. Reitter (3) says that the stem is hollowed out from 2–10 mm. below the buds, in attacks on pears in Germany. The damage which we found below the bud was never more than about 4 mm., and this only very rarely. A few of the infected buds partially open but they are soon killed if the larvae continue to feed. Occasionally small tortrix caterpillars may be found damaging the same buds.

6. NATURE OF DAMAGE.

In the larval stages this pest kills the fruit buds and occasionally the lateral wood buds of the second year's growth of apple trees. We have never found terminal wood buds attacked, but in the cases we have investigated the percentage of buds killed has been very small. Fruit buds may be attacked when they are formed at the terminals.

The larva feeds on the succulent part of the buds and continues to feed until only the bud-scales remain, by which time it is ready to pupate. Each larva thus destroys one fruit bud.

Buds from which the weevils have escaped can easily be recognised as they are hollow, remain on the tree, and have a large lateral hole about 2 mm. in diameter.

In the adult stage (see Plate XVI, fig. 1) we have had little opportunity of studying the damage done by the adult in the orchard, but we have studied the injury they cause when "sleeved" on apple trees from June to February. In the "sleeves" during the summer the weevils caused the leaves to fall prematurely by puncturing the base of the petioles, usually in several places. They also badly injured the young shoots by making holes in them, usually near a leaf, or through the bud-scars at the base of the shoot. A number of buds were found punctured, and also the succulent part of the fruit spurs below the buds.

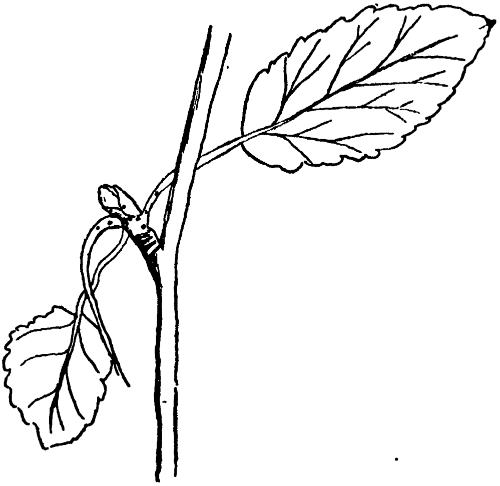


Fig. 10. Apple shoot showing the position of the punctures made by the adults of *A. cinctus*.

After the leaves had fallen, they continued to feed by making holes in the buds: in this way a number of buds were killed, and others were punctured to their centres.

From the above it will be seen that this insect has great potentialities as a pest; but, up to the present, it has only been found in small numbers in this country. Near Cambridge over 90 per cent. of them were killed by external parasites. Near Wisbech no parasites have been found, but here the weevils have been kept down by continuous collecting.

It is important that collectors and growers who find this pest should record its presence, so that steps may be taken to try and prevent it from increasing in this country.

7. PARASITES.

For the purpose of obtaining data on the life-history we collected and examined a number of damaged buds at frequent intervals. About the beginning of May we found a 3rd stage larva being attacked by an ectoparasitic larva, which we took to be *Pimpla pomorum*, and by the third week in May the percentage attacked by this parasite had increased enormously.

An examination of a number of buds on May 18th showed six larvae of *A. cinctus* all parasitised. On May 21st twelve larvae all parasitised were found and also one healthy pupa.

At the same time we examined a number of *Anthonomus pomorum* larvae and found that *Pimpla pomorum* were attacking less than 10 per cent. of these.

In order to determine the periods of the larval instars, we dissected the attacked buds. After dissection we put the parasites back with their host, but most of them died and only five pupated. (No cocoon was found.) Only one adult (a female) emerged, and the wings of this had to be removed and mounted to show their characteristics. This female was identified by Dr Waterston and by Mr G. L. R. Hancock as *Pimpla* (*Epiurus*) *pomorum*, Ratz. (with an unusually dark-coloured abdomen).

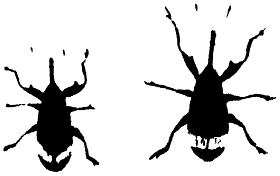
One larva (which was moulting) was preserved from the batch of *A. cinctus* parasites and in this we were unable to see any marked differences from the larva of *P. pomorum*, Ratz. as figured by Imms (9).

So thoroughly did this parasite do its work that we were unable to find specimens of *A. cinctus* during the winter 1923–4. Parasitism to this extent is unusual in this country and in this connection it should be borne in mind that the total number of the hosts (*A. cinctus*) was between 50 and 60 and more than half of these had been removed before the parasite appeared.

8. CONTROL.

Up to the present we have had no opportunity of carrying out experiments on the best means of controlling this pest owing to the small numbers which we have found.

The control measures suggested by the Continental observers are the removal and destruction of the buds which are attacked in the spring



A B

Fig 1.

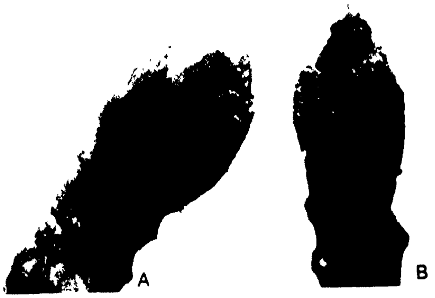


Fig. 3.

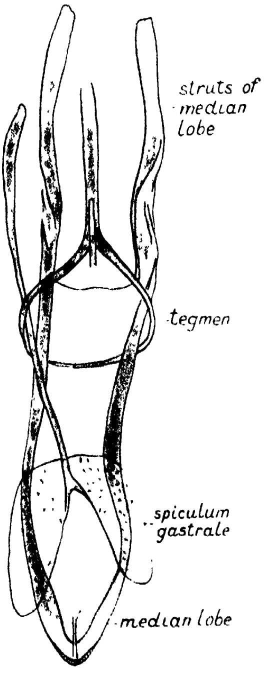


Fig. 2.

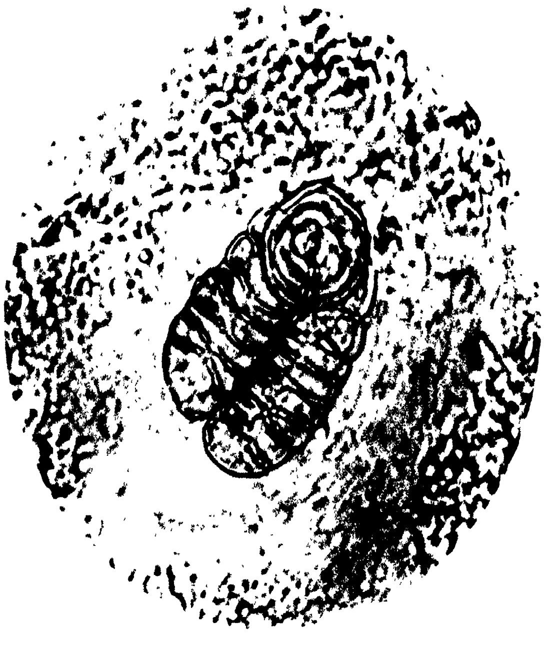


Fig. 4.

PETHERBRIDGE & COWLAND.—The Apple Bud Weevil, *Anthonomus cinctus*, Koll. (pp. 482—497.)

when the normal buds have opened, and at which time the infected buds are very noticeable.

The writers wish to express their thanks to Mr A. W. Rymer Roberts for his criticism and advice.

9. SUMMARY.

Anthonomus cinctus is widely distributed on the Continent and occasionally, in certain districts, does serious damage to pear buds, and now and then is found injuring apples. In this country it is not yet recorded on pears, but was found killing apple buds at Wisbech, in 1921, and near Cambridge, in 1922. Adults have also been found in Kent.

The eggs are laid in apple buds (usually fruit buds) in the autumn, one egg only in each bud. The larva hatches at the end of February and eats out the succulent portion of the bud. It pupates in May and changes to the weevil stage in June. The weevils puncture the leaf stalks, buds and succulent portion of the shoots.

A description of the various stages is given.

A comparison is made between this species and those closely allied.

Near Cambridge *Pimpla pomorum* killed most of the larvae and pupae left on the trees after collecting.

REFERENCES.

(1) CHAMPION and HARWOOD (1921). *Ent. Mo. Mag.* Oct.
(2) BEDEL (1888). *Coléop. du bassin de la Seine*, VI, 296.
(3) REITTER (1916). *Fauna Germanica, Käfer*, V.
(4) BOISDUVAL (1867). *Entom. Hortic.* 150.
(5) TASCHENBERG (1879). *Pract. Insektenkunde*, 153.
(6) VERHOEFF, K. W. (1923). *Arch. f. Naturgesch.* 89, A 1, 45.
(7) DECAUX (1891). *Feuilles Jeunes Nat.* 22, 186.
(8) RITZEMA BOS (1915). *Tijdschr. Entomol.* D 58, xlii.
(9) IMMS (1918). *Ann. App. Biol.* IV, 211.

EXPLANATION OF PLATE XVI.

Fig. 1. A. *A. pomorum.* B. *A. cinctus.*

Fig. 2. Male genitalia of *A. cinctus* (ventral view).

Fig. 3. A. Apple bud containing egg of *A. cinctus* photographed in March, 1923, showing the puncture made by the female before laying the egg. B. Apple bud showing the characteristic exit hole of the adult.

Fig. 4. Second abdominal spiracle of a third stage larva of *A. cinctus*, showing the outline of the chamber and its two sacs. ($\frac{1}{6}''$ objective used.)

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NOTES ON THE BIOLOGY OF *PSEUDOCOCCUS GAHANI*, GREEN

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INTRODUCTION

Pseudococcus gahani, a "Mealy Bug" indigenous to the British Isles, was first described by E. E. Green (1) on Red Ribes, and has since been recorded by him from *Ceanothus* sp., Laburnum, and potato tubers (2). This last record brings this species within the range of the Agricultural Zoologist, and the following observations were made on the material on which this record rests. Briefly the history of this insect as a potato pest is as follows. In December, 1922, Mr H. L. Jones, Horticultural Superintendent for Flintshire, discovered a "Mealy Bug" on seed potatoes at St Asaph, and this was reported by him to Mr C. L. Walton, the Adviser in Agricultural Zoology for North Wales. The potatoes were "King George," and had been placed during the previous July on the window ledge of a small empty greenhouse, but their place of origin and manner of infestation cannot be traced. Specimens of the coccid were sent to Mr E. E. Green, who identified them as *Pseudococcus gahani*, Green.

Subsequently all the infested tubers were removed to the laboratory at Bangor, where the life-history, habits, etc. of the coccid have been investigated. The tubers were very much shrivelled, and before work commenced it became necessary to transfer the stock of coccids to fresh tubers. For this purpose sprouted tubers of Sharp's Express and Great Scot were used. Detailed work was commenced in November, 1923.

LIFE-HISTORY.

Female.

(*a*) The adult female has already been described by Green (*op. cit.* (1)), and those examined agree with his, except for slightly greater length measurement. The average of ten specimens was, length 3·38 mm. and breadth 1·25 mm.

(*b*) Egg and ovisac. The egg is marbled, light brown and pale yellow, with a smooth unsculptured surface 0·38 mm. long and 0·25 mm. broad. It is elongate oval in shape and as many as 127 eggs are deposited in a single ovisac. This latter is usually elongate, about 4·5 mm. long and 1·5 mm. broad, and its shape depends to a large degree upon the surroundings. When formed on a smooth portion of a tuber it is roughly spherical, but when constructed in a groove or wrinkle of the skin, it is elongate. Whatever the shape, it is composed of white threads, closely felted together, which become brittle and ruptured with age.

(*c*) The first stage larva is brown, scantily covered with a white waxy secretion, which gives it a greyish colour. The eyes are situated at the base of the antennae, and are simple ocelli. The six-jointed antennae are 0·17 mm. in length. The inter-antennal margin of the body is lobed. Average length of the body 0·36 mm. and breadth 0·20 mm., length of leg 0·20 mm.

(*d*) In the second stage the antennae are 0·25 mm. long and the legs 0·50 mm. in length, while the body is 1·38 mm. long and 0·70 mm. broad. The inter-antennal margin is still lobed.

(*e*) The demarcation of the body into segments is more pronounced in the third stage, due to the greater quantity of white waxy secretion (except at the folds of the segments). The brown basal body colour becomes visible at these folds, producing a symmetrical pattern. The inter-antennal margin is uniform, there being no lobes as in stages 1 and 2. The loop of the mouth-filaments reaches as far as on a level with the bases of the hind pair of legs (the length of the latter being 0·50 mm.), and only a portion of the tarsi now project beyond the margin of the body. The average body length is 2·2 mm. and the breadth 1·1 mm.

Male.

(*a*) The adult male is a delicate, fragile insect with long antennae, legs and wings. The caudal extremity terminates in a pair of long, slender filaments, and a pair of shorter ones on the outer side. Body greyish-brown, covered with white powdery wax. The slender ten-jointed antennae are liberally covered with setae; the two basal joints, short and stout, the remainder elongate. Eyes in the form of large, simple ocelli are present on both surfaces of the head. In place of the mouth-parts, there is a plate of chitin. The wings are rounded on the outer side and hyaline in colour.

Length of body is 1·2 mm., breadth 0·35 mm.; length of leg 0·65 mm.; length of antennae 1·0 mm. Wing expanse 3·3 mm.

(*b*) The first stage larva is paler in colour than the female first stage larva. The body which is 0·45 mm. long and 0·30 mm. in breadth is covered with white waxy secretion. The inter-antennal margin is not lobed, and body segmentation is indistinct. The antennae are six-jointed, the basal joint stouter than the remainder and 0·25 mm. long. The mouth-parts are situated in front of the anterior pair of legs, and mouth-filaments reach almost as far as the posterior extremity.

(*c*) The second stage larva is of a brownish colour, the mid-dorsal and mid-ventral regions deeper than those at the sides. Length of the body 1·0 mm., breadth 0·50 mm.; length of antennae 0·37 mm., and the leg is 0·57 mm. long.

(*d*) In the pre-pupal stage the male is enclosed within a sac-like puparium, composed of a white, felted, thread-like, waxy substance, which is constructed from front to back, the head region being enclosed first. When extracted and mounted the colour of the insect is marbled, light brown predominating, but some of the white secretion still adheres' giving it a greyish tint. Complete ecdysis takes place in this stage, and a lengthening of the body occurs. Wing-buds in the form of lobes develop on the body-margin between the bases of the first and second pairs of legs. Immediately prior to pupation the body length is 1·15 mm.; breadth 0·60 mm.; length of antennae 0·15 mm.; length of leg 0·25 mm. The abdomen is about one and a half times the length of head and thorax together.

(*e*) The brown-coloured body of the pupa is long (1·20 mm.) and narrow (0·42 mm.), with the general outline of the adult, the leg and antennal sheaths free. The antennal sheaths which reach as far as the second pair of legs are 0·42 mm. long. The narrow wing-sheaths, reaching beyond the last pair of legs, are 0·44 mm. long; pointed at the free end, and translucent, almost white in colour. The penial sheath in this stage is distinct and triangular. Length of leg 0·55 mm.

LIFE-CYCLE.

During warm, sunny days the females are very active, searching continually for their somewhat sluggish partners. Although close vigilance was kept, the writer failed to observe the two sexes pairing. At a mean temperature of 55° F. a female takes 7–9 days to construct the ovisac and lay her eggs. After completing the ovisac (in some instances two sacs are formed, but in such cases both are smaller in size), the female dies, remaining outside the structure, with the elevated posterior portion of the body resting on it. Larvae appeared six weeks after deposition of eggs, the mean temperature during the period being 54·5° F.[1] These remained in the first stage for a month, but one batch remained for two months without moulting. The average period of the second stage is 4–5 weeks, and that of the third stage 6–8 weeks. The adult female attains full growth about a month after the final moult. Thus the whole life-period, from the time the eggs are laid to the construction of the ovisac by females of the new brood, is approximately six months.

Male larvae hatched during the first week in November, within a month after ova had been deposited. These larvae moulted on the 2nd and 3rd of December (mean temperature during the period 53° F.), remaining in the second stage for over a month. On January 5th, 1924, some of these larvae were observed to construct cocoon-like sacs, completing them in 40–48 hours. By opening these cases, and examining the males at definite periods, it was possible to observe the gradual change from the pre-pupal to the pupal stage. This took place in the majority of cases during the second week in February. On the 17th of March one pupa was found to cast the pupal skin, pushing it out at the anal extremity of the puparium. Two males hatched out two days later, and two others on March 26th. From these data it appears that the life-period of the male varies between 20 and 24 weeks.

HABITS.

On hatching from the eggs, the larvae are very active; the majority of them move towards light, and are capable of covering a distance of as much as 6 feet before becoming exhausted. Normally, after 12–18 hours activity they settle down upon the tubers, immediately inserting their long hair-like sucking apparatus into the tissues. When irritated the adult females exude a claret-coloured liquid in round drops, which soon dries resembling a small balloon, as already noted by Gahan (*op. cit.*(1)). The amount of honey-dew secreted by the females is small, only on rare occasions was it noticeable, even after prolonged infestation. Large batches of larvae hatched at various times in the laboratory, from the original material obtained, both before and after work commenced. In some instances great numbers of larvae were crawling about. The males are very sluggish in their habits, and none were seen on the wing. Only with difficulty could they be induced to move.

There seems little likelihood that this coccid will become a troublesome potato pest, for as far as the present observations go, there appears to be only two broods in a year. The life-period is also very extended, each brood taking on the average six months to complete the cycle. Potatoes are seldom stored or boxed for "seed" for more than six months at the most, and would need to be very heavily infested early on to suffer serious damage.

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[1] Ovisacs completed over eight weeks ago, and subjected to a mean temperature of 54° F., contain eggs which are still unhatched at the time of writing.

The work has been carried out in the laboratory of Agricultural Zoology at Bangor University, and I wish to thank Mr C. L. Walton, the Adviser in Agricultural Zoology for North Wales, for suggesting this work and placing the material at my disposal. I also wish to thank Mr E. E. Green for kindly identifying the specimens, for information and suggestions.

SUMMARY.

(1) *P. gahani* has previously been recorded by Green as occurring upon Red Ribes, Ceanothus, Laburnum and potato tubers. This last record is here elaborated.

(2) The female passes through five stages, while the male passes through six.

(3) The life-period in the two sexes is very extended, ranging between 20 and 24 weeks.

(4) Owing to the long life-period no fears should be entertained of this coccid becoming an economic pest.

BIBLIOGRAPHY.

(1) Green, E. E. (1915). *Entomologist's Monthly Magazine.* 3rd S. I, June, 179–180.

(2) —— (1922). A brief Review of the Indigenous Coccidae of the British Islands. *Proc. S. Lond. Ent. and Nat. Hist. Soc.* 22.

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"FRUITINESS" IN WHEY

PART I. BACTERIOLOGICAL STUDIES

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THE following investigation was undertaken to gain some knowledge of the origin of a characteristic "fruity" smell which sometimes develops in whey during the process of cheese making. The particular sample on which this work is based was procured at the Dairy Show of 1920, from whey which had developed a well marked odour.

ISOLATION OF CAUSAL ORGANISM.

Since it was found possible to reproduce this fruity smell by means of direct subculturing from the sample of whey into sterile milk, an attempt was made to isolate the causal organism or organisms. Microscopical examination of the whey showed the presence of cocci, bacilli and yeasts. Tubes of sterile milk were inoculated with about 1 c.c. of the original sample of whey, and incubated at 22° C., 30° C., and 37° C. Each tube showed coagulation of the milk, but the tubes incubated at 37° C. alone showed fermentation and gave the characteristic sweet, apple-like odour. Microscopical examination of this culture showed the presence of numerous bacilli, yeasts and a few cocci. To effect the separation of these organisms, the milk culture was plated out on beer wort agar and whey agar. By repeated plating on beer wort agar, and finally by means of Barber's single cell culture method (2), pure strains of the yeast were obtained. The strain used in the experiments to be described below is specified as *YII*.

The isolation of the bacillus presented greater difficulty, since it did not grow readily on artificial media. The colonies on whey agar were very indefinite, appearing as a "haziness" in the medium. To the naked eye they suggested a flocculent precipitate in the medium, and it was only when examined under the low power of the microscope that they were revealed as masses of tangled threads of bacilli. These colonies readily curdled milk, but would rarely grow when transplanted on whey agar. In the process of isolation therefore it was necessary to pass this organism frequently through milk. A single cell culture was eventually obtained by Barber's method (2), but this was discarded since it proved to be far less virile than the culture, obtained by repeated plating out, which was used in the experimental work recorded and is denoted in the text as *SCV*.

During the process of plating out, in addition to the yeast and the bacillus, strains of cocci and a species of mucor were isolated. A description of the two latter is not included in this paper since it was found that they were not concerned in the production of the fruity odour. This was produced only when the yeast and the bacillus were acting in conjunction in the milk.

DESCRIPTION OF THE BACILLUS.

The bacillus grew well in sterile milk at temperatures from 30° C. to 37° C., with the formation of a firm curd. Examined under the microscope, the milk showed the presence of chains of bacilli, generally long and twisted, frequently capsulated. The bacilli were gram positive, and often showed the presence of granules when stained with methylene blue. Great difficulty was experienced in inducing growth on other artificial media. Whey agar was the only solid medium that gave satisfactory results, and even on this the organism required frequent passage through milk if its vitality was to be maintained. With repeated subculturing even into sterile milk it has at the present time lost much of its original vigour. Because of this aversion to artificial media fermentation tests carried out with litmus peptone water containing various carbohydrates and alcohols were without result. Further tests carried out with casein bouillon and yeast extract as the foundation of the media were rather more successful though still unsatisfactory. The results for these two series are shown in Table I, where the figures given represent the amount of acid, determined by titration with NaOH and calculated as lactic acid, produced in 100 c.c. of the medium.

Table I.

	Grams of Lactic-Acid in 100 c.c. of Medium																
Medium	Glycerol	Xylose	Arabinose	Sorbitol	Mannitol	Laevulose	Dextrose	Mannose	Galactose	Saccharose	Maltose	Lactose	Raffinose	Inulin	Dextrin	Starch	Salicin
Casein Bouillon	0·05	0·11	0·14	0·06	0·06	0·18	0·26	0·18	0·11	0·07	0·11	0·18	0·035	0·05	0·05	0·05	0·05
Yeast Extract	0	0	0	0	0	0·18	0·59	0·20	0	0	0·09	0·50	0·07	0	0·2	0	0

The optimum growth occurred at about 35° C., though feeble growth could still be detected at temperatures as low as 20° C. and as high as 47° C. This bacillus produced in milk large quantities of inactive lactic acid (up to 2·5 grams of lactic acid per 100 c.c. of milk), together with a small quantity of acetic acid. There was also an increase in the amount of amino-nitrogen present.

This bacillus was one of the rod-shaped lactic acid bacteria. In many respects it closely resembled the thermobacteria (cf. the classification of Orla-Jensen (9)) commonly termed *B. bulgaricus*, a name which is generally used to denote a series of similar organisms. A comparison of the characters of the thermobacteria, and of the bacillus *SCV* isolated from the fruity whey, is shown in Table II.

Table II.

	Thermobacteria or *B. bulgaricus*	*SCV*
Growth on solid media	Difficult. Thin at surface	Difficult. Hazy growth
Morphology	Chains, often long and twisted	Chains, often long and twisted
Staining	Gram positive. Granules with methylene blue	Gram positive. Granules with methylene blue
Aerobiosis	Anaerobic conditions preferred	Anaerobic conditions preferred
Minimum temp. of growth	20°–22° C.	18°–21° C.
Optimum „ „	40° C.	About 35° C.
Maximum „ „	50°–52·5° C.	About 47° C.
Acid production ...	Levo, or more rarely, inactive, lactic acid (1·5–2·75 %). Acetic acid	Inactive lactic acid (2·5 %). Acetic acid
Fermentation reactions ...	Capricious	Variable
Alcohols and pentoses	No action	Very slight (cf. Table I)
Maltose and dextrin ...	Not usually, but if maltose, dextrin also	Both slight (cf. Table I)
Saccharose ...	No action	Very slight (cf. Table I)
Action on casein ...	Direct formation of mono-amino acids	Increase in amount of amino nitrogen in milk

The chief points of difference will be seen to be the lower optimum and maximum temperatures of growth of *SCV* and the slight variations in the fermentation tests.

DESCRIPTION OF YEAST.

The yeast was a Saccharomycete. Spores were produced, though not abundantly, in two to three days at 22° C. The asci were elliptical and contained one to four spores. The vegetative cells reproduced themselves by budding, generally at both ends. The cells in a young culture were typically oval, varying in size from 5·5 × 6·4μ to 9·1 × 10·9μ, but round cells were also frequently present. In older cultures a great variety in size and shape was noted, a large proportion of the cells being sausage-shaped. These cells were much larger than the normal, and tended to hang together in chains. This tendency was most marked in cultures in beer wort which had been kept at a temperature of 22° C. This yeast was able to grow in beer wort from temperatures of 3° C. to 45° C., with fermentation and formation of scum from 11° C. to 45° C. This scum first developed as a ring at the surface, round the sides of the tube, and later formed a waxy covering over the surface. On agitation it was readily broken and sank to the bottom of the tube. The formation of the scum was most rapid at temperatures between 22° C. and 30° C., when it made its first appearance in seven to nine days, and completely covered the surface in about fourteen days. It consisted largely of chains of sausage-shaped cells showing a marked tendency to mycelium formation. This tendency was much less at temperatures above and below these limits. At very low temperatures there was neither scum formation nor fermentation and the cells were generally pear- or club-shaped and showed little tendency to hang together in colonies.

On beer wort agar it produced a heavy cream-coloured growth which later became brown and somewhat "granular" in appearance. Colonies on beer wort agar were cream-coloured, opaque and hemispherical. In stab cultures in beer wort agar and sugar gelatine, there was growth throughout the stab and also good growth on the surface. In one instance in which such a stab culture in sugar gelatine was kept for three months in a cold room, the growth on the surface was found to consist of cup-like erections composed of chains of long irregular cells and small round ones in groups. In the depth of the medium the growth appeared as a succession of discs with feathery edges. These consisted of chains (often branching) of elongated cells, with groups of smaller cells, showing on the whole a marked resemblance to a mycelium of *Monilia.*

The yeast was able to grow in milk, but caused no apparent change. Fermentation occurred in solutions containing saccharose, glucose, fructose and galactose, but not in lactose.

Since this yeast forms spores it must be classed among the *Saccharomycetes*, and since it reproduces itself by budding, and sets up alcoholic fermentation in sugar solutions, it must be placed in the genus *Saccharomyces.* In this genus it belongs to the sub-group which ferments dextrose, maltose and saccharose, but not lactose, a sub-group which includes most of the yeasts used in the making of wines and beers. Amongst these it most closely resembles *S. ellipsoideus*, but differs markedly from it in that it does not readily form spores.

As the work progressed it became clear that the fruity odour developed only when the bacillus and the yeast were both present in the milk. It also became evident that the bacillus was capable of fermenting lactose, but the yeast, although it readily fermented glucose and galactose, was without action upon lactose. Hence it seemed reasonable to suppose that the bacillus functioned by converting the lactose into simpler sugars, which were then available for fermentation by the yeast with the production of the characteristic odour. A study of the chemistry of the reactions concerned in the production of the fruity whey is included in Part II.

PART II. CHEMICAL STUDIES

BY E. R. HISCOX, M.Sc.

Since the production of the fruity odour was apparently due to the combined action of the bacillus *SCV* and the yeast *YII* on the milk (Part I), it was thought to be of interest to identify the substance or substances to which the characteristic odour was due, and to study the chemical reactions involved.

The bacillus was inoculated into sterile milk and allowed to grow until a clear whey could be separated from the curd. Tests carried out on this whey confirmed the view that the acid produced was mainly, if not wholly, lactic acid.

A mixed culture of the bacillus and the yeast was next allowed to grow in raw and sterile milk and whey. The characteristic odour readily developed and when the products were distilled in steam, the vapours being collected in water contained in ice-cooled receivers, the distillates possessed in a more or less marked degree the characteristic odour sought, and gave indications of the presence of an aldehyde, ethyl alcohol, and a trace of acidity.

A series of more detailed analyses of cultures in sterile milk[1] of the bacillus, the yeast, and the bacillus and yeast combined, was undertaken in order to study their action, singly and jointly, on the milk. The analyses were carried out in the following way:

---

[1] The milk used in these experiments was sterilised by heating in the autoclave at 115° C. for 15 mins. The sterility of the milk was tested by subsequent incubation at 30° C. for not less than 10 days.

The titratable acidity. This was determined by titration with $N/4$ NaOH solution and estimated as lactic acid.

Sugars. The sugar was estimated as lactose, by means of Fehling's solution, after precipitation of the protein with $CuSO_4$ and KOH (Ritthausen (5)). In order to determine whether the sugar was purely lactose, or whether glucose and galactose were also present, the sugar in a further sample was hydrolysed by heating for 30 minutes at 115° C. with 4 per cent. H_2SO_4. The sugar in the neutralised solution was determined as above. For pure lactose the reducing power is increased by 40–43 per cent. (8) by hydrolysis. A lower percentage denotes the presence of simpler reducing sugars.

The volatile acidity was estimated by steam distilling a portion of the milk culture, and titrating the distillate with $N/4$ NaOH solution. The nature of the volatile acid was determined with the aid of Duclaux' numbers (4). In this case it proved to be practically pure acetic acid.

Alcohol. The alcohol was detected by means of the iodoform test and Klöcker's modification of Pasteur's drop reaction (6). In those cases in which quantitative determinations have been made, the steam distillate was re-distilled into a pyknometer, and the amount of alcohol estimated from the specific gravity of this liquid.

Aldehyde in the distillate was detected by its peculiar odour, and by the yellow colour and characteristic smell of aldehyde-resin which developed on boiling with KOH. In the later stages of the work the delicate and practically specific test for acetaldehyde, by means of sodium nitroprusside and piperdine (Rimin's reaction—Lewin's modification (10)), was almost exclusively used. Quantitative determinations were made by shaking the distillate with a solution of sodium bisulphite and estimating the residue of bisulphite solution by titration with standard iodine solution.

FIRST SERIES.

Flasks containing 500 c.c. of sterile milk were inoculated with the bacillus, the yeast, and a mixture of the bacillus and yeast, respectively. Parallel cultures were made in sterile milk containing an excess of $CaCO_3$. The cultures were incubated at 30° C. for five days, and then stored in a dark cupboard at room temperature until analysed—5 to 10 days in the first case, 20 to 30 days in the second. During the period of incubation the flasks containing the $CaCO_3$ were frequently shaken to prevent the accumulation of acid. In these circumstances it was not possible to determine the titratable acidity, and it was necessary to add H_2SO_4 to the milk to liberate the volatile acid before the distillation in steam.

The results of the analyses are given in Table III:

Table III.

	Culture	Total titratable acidity as lactic acid gms. / 100 c.c. milk	Volatile acidity as acetic acid gms. / 100 c.c. milk	Sugars as lactose gms. / 100 c.c. milk	Increase in reducing power after hydrolysis %	Alcohol	Aldehyde
1	Milk - Control	0·204	–	5·400			
2	Milk + *YII*	0·214	–	5·218		–	–
3	Milk + *SCV*	2·54	0·04578	3·074	25·5	–	–
4	Milk + *SCV* + *YII* ...	2·347	0·03993	0·984		+	+
5	Milk + $CaCO_3$ - Control ...		0·0079	5·212	38·7	–	–
6	Milk + $CaCO_3$ + *YII* ...		0·0113	5·134	36	–	–
7	Milk + $CaCO_3$ + *SCV* ...		0·1092	0	–	–	–
8	Milk + $CaCO_3$ + *SCV* + *YII*		0·1094	0	–	+	+

From this table it will be seen that the yeast, whilst able to grow in milk, had no apparent action upon it, though probably a very small quantity of lactose was used for purposes of assimilation. The bacillus, on the other hand, had a considerable action. A large quantity of lactic acid was produced together with a small quantity of acetic acid. Almost half the sugar was consumed, and since the increase in the power of reduction after hydrolysis was 25·5 per cent., it may be assumed that the lactose was converted into glucose and galactose by the action of the bacillus. When the bacillus and the yeast were both present a very active fermentation took place with the production of alcohol and aldehyde. The two organisms acting in conjunction were able to remove practically the whole of the sugar even when $CaCO_3$ was not present. The small residue may have been embedded in the hard lumps in the curd, and thus would not have been available to either organism.

SECOND SERIES.

It was thought that probably the amount of aldehyde produced could be increased if the conditions were made more favourable for the action of the yeast, *e.g.* by the addition of glucose to the milk. Table IV gives the record of the results of the analyses of a series of cultures of the organisms in milk and milk to which glucose had been added, undertaken primarily to compare the aldehyde production under these conditions.

In this table an attempt has been made to denote the sugar as quantities of lactose and glucose. This has been done by assuming that the increase in reducing power after hydrolysis is exactly 40 per cent. for lactose. The figures given are, therefore, only approximate. The high percentage increase in No. 5 was probably due to the fact that the glucose used was "Commercial" and contained dextrin.

Table IV.

	Culture	Total titratable acidity as lactic acid gms. / 100 c.c. milk	Volatile acidity as acetic acid gms. / 100 c.c. milk	Sugars		Increase in reducing power after hydrolysis %	Alcohol gms. / 100 c.c. milk	Aldehyde gms. / 100 c.c. milk
				Lactose gms. / 100 c.c. milk	Glucose gms. / 100 c.c. milk			
1	Milk – Control	0·204	–	5·214	–	41·5	–	–
2	Milk + *SCV*	2·41	0·03828	1·374	0·798	25·8	–	–
3	Milk + *SCV* + *YII* ...	2·100	0·0245	0·502	–	77·4	+	0·1118
4	Milk + glucose – Control ...	0·259	0·0184	5·214	3·124	19·6	–	–
5	Milk + glucose + *YII* ...	0·349	0·0175	5·428	–	53·3	1·34	Trace
6	Milk + glucose + *SCV* + *YII*	2·57	0·0513	2·00	–	87·0	+	0·1720

From the table it is seen that there was a slight increase in the amount of aldehyde produced when glucose was added to the milk, although the sugar was not entirely consumed. The vapours which passed over at the beginning of the distillation had an unmistakable odour of acetaldehyde.

The yeast fermented the whole of the glucose with almost quantitative production of alcohol, leaving the lactose practically untouched.

The results of these analyses confirm the hypothesis that the bacillus functioned by converting the lactose into sugars which were available for fermentation by the yeast. The fruity odour was due to the products of this fermentation, namely, acetaldehyde and alcohol, admixed with acetic acid produced by the action of the bacillus.

THIRD SERIES.

Further experiments were carried out to test whether the presence of both organisms in the living state was essential for the production of the characteristic fruity odour. The bacillus was inoculated into sterile milk and allowed to grow. At the end of about a month the culture was subjected to a temperature of 100° C. for 30 minutes. Inoculations were made from this heated culture into milk in order to ascertain that the bacillus had been killed. A further sample was titrated and distilled in steam. The distillate showed the presence of volatile acid, but no trace of alcohol or aldehyde. The residue of the milk culture was inoculated with the yeast, and incubated at 30° C. The characteristic smell developed, and when the culture was analysed, the titratable and volatile acidities of the milk were found to be practically unchanged, but alcohol and acetaldehyde were present in the distillate.

Analyses of cultures of the yeast in milk + lactic acid + glucose; milk + acetic acid + glucose; milk + glucose; peptone water + glucose; water + glucose, readily demonstrated the presence of alcohol and acetaldehyde in each case, although there was only a trace in the culture in water + glucose, probably due to scanty growth and fermentation. Thus it would seem that the glucose was directly concerned in the production of the acetaldehyde.

Neuberg (7) has recently shown that acetaldehyde is formed as an intermediate product in the degradation of glucose during alcoholic fermentation, and that it can be retained and detected in the solution if $NaHSO_3$ has been added. More recently Abderhalden (1) has demonstrated that it can be adsorbed by certain colloids present in the medium. On the other hand, acetaldehyde may be formed by the direct oxidisation of ethyl alcohol.

In order to test which, if either, of these reactions was responsible for the production of the acetaldehyde, two flasks of sterile milk were inoculated with the yeast and the bacillus. One was placed in a jar under anaerobic conditions (coal gas—exhaust—pyrogallol), the other under ordinary aerobic conditions. Both flasks were incubated at 37° C. for three days, by the end of which time the milk had coagulated and a vigorous fermentation set in. They were then removed to an incubator at 14°–16° C., and at the end of 14 days the contents of both flasks were distilled in steam. Each distillate contained ethyl alcohol, showing that alcoholic fermentation had taken place, but in that from the culture which had been grown under anaerobic conditions no trace of acetaldehyde could be found although the distillate from the aerobic culture gave a definite reaction.

The theory that the production of the acetaldehyde was the result of oxidisation of the alcohol was confirmed by placing the yeast in 1 per cent. alcohol solution. Under ordinary aerobic conditions, at a temperature of 14°–16° C. acetaldehyde could be detected in the solution within a few hours. Under anaerobic conditions (as described above), no acetaldehyde could be detected at the end of 14 days, but when this sample was returned to the incubator under aerobic conditions, acetaldehyde was formed within 24 hours.

It was thought of interest to study the interaction of the yeast and other lactic acid bacteria commonly present in milk and milk products. *B. bulgaricus*, which is known to hydrolyse lactose (3), gave, in combination with the yeast, a vigorous fermentation, with good production of acetaldehyde. Combinations of two strains of "starter" bacteria and the yeast, and of *B. coli* and the yeast, gave negative results.

Of six unidentified, different strains of yeast tested for their action upon 1 per cent. alcohol, four readily produced acetaldehyde. One of these, a lactose fermenting yeast isolated from a "blown" cheese, produced acetaldehyde when inoculated into sterile milk.

By the courtesy of Mr Todd, a number of cheese were made at the British Dairy Institute under the supervision of Mr Todd and Mr Holmes, from milk which had been inoculated with these organisms, in order to test their influence on the market value of the cheese. The milk used was of low bacterial content, and all utensils were carefully steamed before use. The quantity of milk and the amount of space available at the time were limited. The type of cheese chosen was therefore the Smallholder, which is quickly made and is small in bulk. Moreover, the curd is scalded at a high temperature, and is comparatively slightly pressed, conditions which should be favourable to the growth of the bacillus and subsequent action of the yeast, respectively. On the other hand the short time during which the high temperature was maintained tended to limit the growth and consequent action of the bacillus.

The bacillus *SCV* originally isolated from the fruity whey had at the time become very slow in its action. A vigorous culture of *B. bulgaricus*, which hydrolysed lactose and brought about the same reaction in combination with the yeast, was therefore substituted for it in these experiments. In two batches of cheese *B. bulgaricus* was used as a starter, in a third a mixture of *B. bulgaricus* and a pure culture of a "starter," and in the fourth an ordinary dairy "starter" only.

The yeast was added to the milk after it had been placed in the vats. Control cheese were made in each case without the addition of the yeast. During the making of the cheese a distinct fruity odour was noted in the vats containing *B. bulgaricus* and the yeast, particularly after the cutting of the curd. In all other cases it was entirely absent. When the cheese were ripened, those containing *B. bulgaricus* and the yeast had developed a pronounced fruity flavour, and alcohol and acetaldehyde could be detected in the steam distillates from these cheese. The cheese containing the ordinary dairy starter and the yeast was very slightly "yeasty," but the characteristic fruitiness was not present, neither could acetaldehyde be detected in the steam distillate. The control cheese were normal in flavour.

Summary.

1. A case of "fruitiness" in whey was found to be due to the interaction of a bacillus and a yeast.
2. The bacillus, during fermentation, hydrolysed the lactose with the production of glucose and galactose.
3. The yeast was without action on lactose, but readily brought about alcoholic fermentation of the products of the bacterial hydrolysis.
4. The fruity odour was due to acetaldehyde admixed with alcohol and acetic acid.
5. The acetaldehyde was produced by oxidisation of the ethyl alcohol by the yeast.
6. The "fruitiness" was reproduced in cheese containing *B. bulgaricus* and the yeast.

This work was carried out at the National Institute for research in Dairying, and at the Royal Technical College, Copenhagen. I wish to express my thanks to Professor S. Orla-Jensen for much valuable help and encouragement, and to Professor Søncke Knudsen for his kind interest and valuable suggestions.

REFERENCES.

(1) Abderhalden, E. (1921). *Fermentforschung*, v. 2.
(2) Barber, M. A. (1914). *Philippine Journal of Science*, ix. 4.
(3) Bertrand, G. and Weisweiller, G. (1906). *Ann. Inst. Pasteur*, 20.
(4) Duclaux, E. *Traité de Microbiologie*, iii. p. 384; iv. p. 685. (Masson & Cie, Paris.)
(5) Fresenius (1878). *Ztschr. f. analyt. Chem.* p. 241.
(6) Klöcker, A. (1911). *Centralbl. f. Bakt.* ii. 31.
(7) Neuberg, G., Hirsch, J. and Reinfurth, E. (1920). *Biochem. Ztschr.* 105.
(8) Orla-Jensen, S. *The Lactic Acid Bacteria*, p. 96. (Høst & Son, Copenhagen.)
(9) —— *The Lactic Acid Bacteria*, pp. 160–165.
(10) Stepp, W. (1922). *Biochem. Ztschr.* 127, 1–6.

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REVIEWS

Colour-changes in Animals. The Pigmentary Effector System: A Review of the Physiology of Colour Response. By Dr LANCELOT HOGBEN. (Biological Monographs and Manuals. Pp. xi + 152. Edinburgh and London: Oliver and Boyd, 1924. 10*s.* 6*d.* net.)

Whilst every student of zoology and many physiologists will be grateful to the author for this attractive volume on the physiology of colour-response in Vertebrates and Crustacea, it would be a mistake to imagine that Dr Hogben treats generally of the biology of animal pigmentation. He has selected a definite problem—the factors that determine alteration in the mobile pigment-cells that give colour to the skin—and leaves untouched the wider problems of animal chromatology. The cells that exhibit pigmentary expansion and contraction are accepted as mechanisms responsive to stimulation and are regarded as forming one of the trinity of "effector systems" along with muscle and cilia. We are not told whence they come in development nor is the biochemistry or the physiology of pigmentation discussed. Dr Hogben has no doubt done the right thing by confining this book to the restricted problem but we hope that the editors of the series of which this volume is the first will provide another dealing with the wider aspects of that pigmentary system whose regulation is here described by one who has taken an active part in its investigation.

The pioneer in the work that has led up to our present knowledge of radiation and retraction of mobile pigment was Lord Lister, who with true prophetic insight drew attention in 1858 to the value of the cutaneous pigmentary system of the frog not only as affording insight into the biology of animal colour-change but as giving test-objects for investigation of pharmacological effects, thereby anticipating by some sixty years the modern use of chromatophores as indicators of the action of drugs. The idea of the term "effector system" as applied to the variable pigmentary system may indeed be traced to Lister's famous paper where he says "the pigmentary system also promises to render good service to toxicological enquiry. Hitherto in experiments performed on animals for that object, attention has been directed chiefly, if not exclusively, to the effects produced upon the action of the nervous centres, the nerves and the muscles. In the pigment-cells we have a form of tissue with entirely new functions which though apparently allied to the most recondite phenomena yet produce very obvious effects...and the effects produced are so obviously indicated by the changes in colour of the integument that I venture to recommend this method of investigation to those who are occupied in studying the action of poisons."

The development of endocrinology eventually led to the adoption of Lister's suggestion in a modified form. Adrenalin was shown to produce general contraction of the dark pigment in the skin of frog by Corona and Moroni in 1898, and removal of the pituitary was seen to be followed by extreme pallor in the experiments on tadpoles made by various physiologists between 1914 and 1919. But it was only in 1921 that Hogben and Harley in England and Swingl in America performed the crucial experiment of testing the effects of pituitary extract on the larvae of newts and of the frog and showed that when taken from the posterior lobe of this organ (of any vertebrate) it causes maximum darkening of the skin of frogs. This rapid and general reaction naturally raised doubts as to the accepted view that the pigmentary system in these animals is controlled by the nervous system. The complexity of the nervous control of the vascular system—that system through which the pituitary hormone exercises its influence upon the pigment-cells—was sufficient, when fully realised, to call for the most careful analysis of any colour-effects that were associated with nervous stimulation in the frog, especially in view of the fact that no indubitable nerve-endings in these pigment-cells had ever been demonstrated. Doubts of the reality of any effective nervous control have hardened as more work has been done on the Amphibia and the conclusion reached by Dr Hogben is that the colour-changes of these animals are mainly, if not exclusively, responses to a pituitary hormone the secretion of which fluctuates in correspondence with the action of natural stimuli. Whilst recognising that this correspondence involves an unsolved mystery, the colour-changes of Amphibia may be regarded as hormonic.

The case of fishes shows how dangerous it is to generalise from the phenomena offered by a single order of animals. The colour-changes of certain Teleosts and of Dipnoi offer some of the most rapid and striking cases in the whole animal kingdom and it would have added to the interest of Dr Hogben's work if an account of these colour-effects as seen under natural conditions had been given. The evidence taken from experimental work is however fully discussed and reveals many interesting points that differentiate the chromatophores of fishes from those of Amphibia. Direct evidence for nerve-endings and nervous control has been obtained by Ballowitz and Ponchet. The preparations made by Ballowitz were seen by the reviewer in 1893 and were even more convincing than the excellent figures of his classical paper. But the control of colour-response in fish is not nervous only. Some evidence is forthcoming that as in Amphibia internal secretion plays an important part in the initiation and regulation of the display but in an opposite sense. Pituitary extract, for example, induces contraction and consequently pallor and not expansion or darkening of the skin as is the case in Amphibia. The predominant controlling factor in fish, however, appears to be direct sympathetic nervous action.

The case of reptiles shows a third class of controlling influences; adrenal secretion. The chapter in which Dr Hogben discusses the reactivity of Reptiles involves a previous knowledge of Langley's work on the autonomic nervous system that most of his readers will not possess and the terms used for the action of drugs might have been explained but the general results are (as is always the case with investigations on this little-known order) not very clear. Light may have a direct action on the chromatophores of the skin. Excitement (through adrenal secretion) produces pallor. Pituitary extract appears to have no effect. This section on Reptilian colour-change is a valuable *résumé* and the only omission we have noticed is that Schmidt's important monograph though quoted is not given in the list of literature.

The concluding chapter gives a critical account of the colour-responses exhibited by Crustacea. To one who has taken part in this work, it is of the greatest interest to find comparative physiology coming into its own at last and bringing to bear upon the problems of coloration in *Hippolyte* the new ideas and new methods of interpretation that have been gained mainly in the pursuit of Vertebrate physiology. These prawns appear to live in closer relation with their external environment, whereas the metachromatic Vertebrates are greater humorists. Their chromatophores possess a direct basal reactivity to light and darkness and also an indirect nervous reactivity determined by the direction in which light enters the eye after leaving the background. The former basal control has become fixed as part of the periodic daily rhythm—night phase of activity and day phase of rest—that is exhibited by the whole organism and all its organs. Whether in addition to this, there is also in these prawns an internal humoral regulation analogous to that of Vertebrates, we do not know, but if it exists it appears to play a negligible part in the control of the pigmentary system. More important still is the question of the relation of the chromatophores of Crustacea to nutrition. Since Gamble and Keeble published their evidence on this subject in 1906, the fat-soluble vitamin problem has arisen and the attention of investigators has been mainly directed to the case of Vertebrates and especially of Rats. In *Hippolyte* there is a very suitable object for testing the significance of mobile lipochrome pigment in its relation both to the presence of fat in light and to the absence of fat in darkness. The hypothesis tentatively advanced in 1906 of a photosynthetic action of lipochrome pigment in these animals is, we are glad to see, referred to in this work and it would have been an advantage to future workers in this unexplored field, if this rather crude hypothesis had been considered in the light of the economics of fat-soluble vitamins especially as *Hippolyte* ranges into the Arctic seas.

This volume is a sign of the *rapprochement* between zoology and physiology and forms the first of a series that will be welcomed by all biologists. It contains a finely critical appreciation of the factors governing colour-response and shows not only what is known but what the next problems are and how they may be approached. The author, the editors and the publishers are to be congratulated on the successful launching of such a timely aid to biological research.

F. W. GAMBLE.

Outlines of Fungi and Plant Diseases for Students and Practitioners of Agriculture and Horticulture. By F. T. BENNET. Macmillan & Co. 1924. 7*s*. 6*d*.

This volume consists of a series of extended lectures with practical exercises, is written in an old-fashioned style and is not particularly up-to-date. Part I consisting of sixty-seven pages purports to be an "introduction to the study of Fungi in general" but is distinctly lopsided, for whilst Lichens have ten pages devoted to them, the Rusts and Smuts are not even mentioned. Furthermore it contains much to cavil at. "A structure consisting of living cells only" is not an adequate definition of a thallus: *Pythium de Baryanum* is by no means so common as suggested; to speak of the "*species*" *Penicillium glaucum* is misleading as is also to describe yeast as an anaerobic organism; whilst the author's views on fungus phylogeny need widening. Part II which deals with plant diseases and the fungi which cause them is perhaps better than Part I but is equally in need of critical revision. The inverse relation between vigour of host and incidence of disease is by no means so clear as described nor can much weight be given to the argument that

"Cultivated plants are of more delicate constitution" than wild plants. There is at the time of writing no published evidence of the very important statement on page eighty-six regarding the infection of immune potatoes by *Synchytrium endobioticum*, and it is still premature to class Virus Diseases along with diseases of known bacterial origin. Many of the illustrations might be improved whilst the method followed in referring students to literature is incomprehensible. The volume is, however, a brave attempt to fill a noteworthy gap in English botanical literature and should a second edition be called for a drastic revision would produce a very useful book.

The Evolution of Plants. By DUCKENFIELD HENRY SCOTT. Home University Library. Williams & Norgate, 1924. 2*s*. 6*d*.

A reprint of Dr Scott's rather wonderful little book first published in 1911 and now re-issued at, alas! two and a half times its original price. The volume is unique in its breadth of view and clarity of vision and is still quite the best introduction to the study of the evolution of plants. It seems a pity therefore that Dr Scott should not have produced a new edition incorporating the main results and points of view arising from the work of the past decade. Fortunately these researches can be found put into fine and simple perspective in Dr Scott's *Extinct Plants and Problems of Evolution* published recently.

An Introduction to the Study of Heredity. By E. W. MACBRIDE. Home University Library. Williams & Norgate, 1924. 2*s*. 6*d*.

In the popular view the study of heredity has come to be synonymous with Mendelian investigation and Professor MacBride has done good service in writing the present interesting volume putting the subject in a truer perspective. The book is a vigorous case for Lamarckism and a criticism of the efficacy of natural selection as the originator of new species and of any great light to be thrown on the problem of evolution by Mendelism. The chapters are somewhat unequal but that on the inheritance of sex is a masterly summary. Professor MacBride's appreciation of Tornier's researches is interesting but it is doubtful if their better knowledge will prove quite so revolutionary as the author thinks. The last chapter deals with the laws of heredity on human affairs and this is the weakest portion of the book. The author is a "race dogmatist" and accepts crude ideas of racial psychology perhaps a little too easily—his description of the shuddering Nordic taxpayer is distressing.

The Biological Foundations of Society. By ARTHUR DENDY. Constable & Co. 1924. 7*s*. 6*d*.

Professor Dendy's book is the outcome of a series of lectures delivered at King's College and if the lectures were followed by discussions one can only regret whole-heartedly that one was not present. The work is largely an account of the structure and organisation of the living unit, its hereditary and environmental relationships and its social evolution. Interwoven with the strictly biological argument is a running commentary of sociological observation which one will think foolish or wise according to one's political or social opinions. So closely are the biological facts and sociological hypotheses interwoven that many readers may feel that the latter have taken the reins into their own hands and tended to guide the interpretation of the former. Thus Professor Dendy's naïve psychology, his by no means progressive ideas of the relation of Capital and Labour, Communism and the House of Lords, Birth control and Slum populations, tend to run away with him and lead him into somewhat questionable analogic chains of reasoning. All this is, of course, helped by the author's Lamarckian views which are very apparent throughout. Here and there it is a little difficult to take Professor Dendy quite seriously, as, for example, when he quotes apparently with approval Gates' account of the girl whose ancestors had worn ear-rings and who was born with perforated ear-lobes! On human racial matters Professor Dendy speaks perhaps a little more dogmatically than the evidence warrants, and many of his views on social and national problems are open to criticism. Professor Dendy has, however, written a very interesting book on an aspect of Applied Biology far too neglected by Biologists, and, that one differs from him in perhaps most of his points of view and applications, is only evidence of the book's stimulating quality.

WM. B. BRIERLEY.

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